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RELATION OF HARDNESS AND OTHER FACTORS TO PROTEIN CONTENT OF WHEAT

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It is well known that hard spring and hard winter wheats are higher in protein content than the soft wheats. Since the former are uniformly higher in protein content, it has been assumed that the factors for hardness and protein content are correlated.

As early as 1904 Harper and Peters (*2*)[1] made the following observation regarding wheats used in their experiments:

> The dark flinty kernels are distinctly harder than the light starchy ones and contain more protein.

Snyder (7) reported the analyses for protein of 63 different varieties of wheat, in which each variety was divided into two samples, one containing only light-colored (starchy) and the other only dark-colored (flinty) grains. No hardness test was made directly. The dark kernels were found to contain 15.13 per cent protein as compared with 12.76 per cent for the light-colored kernels. This is a difference of 2.37 per cent in favor of the former.

In another test (*8*) six different samples of wheat were divided into starchy and flinty kernels and analyzed for protein. The flinty kernels contained 14.04 per cent of protein and the starchy kernels 12.12 per cent. In another lot of 12 varieties the starchy kernels contained on the average 12.86 per cent protein and the flinty kernels 14.91 per cent. Averaging all of Snyder's results, there is a difference of 2.11 per cent in favor of the flinty kernels.

Snyder (*8*) also reported the analyses of starchy and flinty kernels of other grains. In all cases the flinty kernels contained the most protein, the difference being 0.82 per cent for corn, 0.75 per cent for rye, and 0.49 per cent for oats.

Shepard (*6*), of the South Dakota Agricultural Experiment Station, reported a protein content of 15.6 per cent for durum wheat and 13.68 per cent for common wheat.

[1] Reference is made by number (italic) to "Literature cited," p. 521–522.

Because of the assumed correlation between hardness and protein content, the commercial grading of wheat and the improvement of wheat by selection is based to a considerable extent on the hardness of the grain. The investigations reported in this paper had reference to improvement in the quality of wheat by selection. Since the protein content of the grain is one of the chief factors that determine quality, the question which formed the central object of this investigation was whether a method of selecting for protein content could be devised that would not involve the necessity of making chemical analyses at first, where hundreds of strains or races of wheat are being tested.

It is evident that, if hardness and protein content are genetically interdependent, this fact can be proved by means of the coefficient of correlation. It would not be possible, of course, to demonstrate the linkage of factors for any individual case. Likewise a parallel association of hardness and protein content might exist without any genetic relationship, so that a positive coefficient would not of itself prove such a relationship but would simply suggest the possibility of its presence. On the other hand, a negative coefficient would preclude such a supposition.

For the purpose of the investigation, 94 pure strains of wheat, each the product of a single head, were chosen, and the correlation coefficient between hardness and the protein content was calculated. Hardness was expressed in terms of the crushing point. The method of determining the crushing point has been described elsewhere (*3*).

For this determination 500 grains were used, it having been determined that this number gives accurate results. The analyses for protein were made by the department of chemistry of the Kansas Agricultural Experiment Station. The crushing points and percentages of protein are given in Table I

TABLE I.—*Crushing points and protein content of 94 pure strains of Kansas-grown wheat*

Serial No.	Variety.	Crushing point.	Protein content.
		Gm	*Per cent.*
909	Fuchs	3,902	11.57
812	Griechischer Sommer von Volo	4,260	12.24
895	Touzelle	5,018	15.52
839	Fenton	5,313	11.48
659	Blanc à duvet velouté	5,407	11.68
980	North Allerton	5,702	10.24
891	Duroselle	6,009	10.60
864	Jones Winter Fife	6,309	12.04
865	....do	6,319	10.96
943	Bastard	6,328	1.36
871	Urtoba	6,340	10.97
1117	Turkey	6,401	13.20
1371	U. S. Cereal Investigation No. 1656	6,410	10.68
1143	Japanese Velvet Chaff	6,494	10.72
886	Japhet	6,516	10.48

TABLE I.—*Crushing points and protein content of 94 pure strains of Kansas-grown wheat*—Continued

Serial No.	Variety.	Crushing point.	Protein content.
		Gm.	*Per cent.*
781	An Australian hybrid	6,523	12.28
890	Duroselle	6,588	11.28
859	Banat	6,662	12.48
915	Paine's Defiance	6,674	11.68
852	Shirreff's Square Head	6,712	10.21
887	Japhet	6,724	10.56
1107	Kharkof	6,894	12.36
801	An Australian hybrid	6,947	12.65
802	....do	6,950	12.32
724	Kubanka	7,086	10.24
1162	U. S. Cereal Investigation No. 1787	7,266	12.88
972	Talavera	7,496	11.36
854	Shirreff's Square Head	7,578	12.00
1159	Turkey	7,633	12.00
764	Crimean (U. S. Cereal Investigation No. 1433)	7,657	11.72
1000	Hickling	7,825	10.96
1115	Turkey	8,021	12.36
778	An Australian hybrid	8,064	13.88
694	U. S. Cereal Investigation No. 1665	8,084	12.28
1149	Japanese Square Head	8,295	11.64
842	Fenton	8,350	12.62
191	An Australian hybrid	8,504	12.26
860	Banat	8,545	12.16
1082–2	Turkey	8,719	12.12
1157	....do	8,884	12.00
882	Japhet	9,078	12.80
977	Spalding's Prolific	9,215	11.20
973	Talavera	9,225	12.62
837	Tagenrog	9,234	11.12
761	Crimean (U. S. Cereal Investigation No. 1435)	9,242	11.36
762	....do	9,391	11.60
893	Duroselle	9,395	10.60
1372–5	Spelt	9,484	11.84
773	An Australian hybrid	9,535	11.80
725	Kubanka	9,617	11.64
754	Beloglina	9,682	10.92
927	California March	9,756	12.08
711	U. S. Cereal Investigation No. 1654	9,825	11.08
772	An Australian hybrid	9,956	11.72
800	....do	9,961	12.60
1116	Turkey	10,010	11.52
1081	....do	10,016	12.52
1064	Macaroni	10,076	11.76
861	Aegyptischer	10,162	12.08
735	———	10,176	11.36
813	Griechischer Sommer von Volo	10,188	12.73
1105	Banat	10,251	11.92
1098	———	10,458	11.35
732	———	10,522	11.30
707	Red Winter	10,546	11.32
929	California March	10,592	11.65
1119	Bacska	10,594	10.94
706	Red Winter	10,645	10.89
734	———	10,765	11.36
1150	Turkey	10,776	12.00
150	Beloglina	10,899	11.67
1128	Crimean	10,855	10.84
752	Beloglina	10,972	13.04
1103	Diehl's Mediterranean	11,017	10.60
940	Bastard	11,309	11.42

TABLE I.—*Crushing points and protein content of 94 pure strains of Kansas-grown wheat*—Continued.

Serial No.	Variety.	Crushing point.	Protein content.
		Gm.	*Per cent.*
722	Ghirka	11,323	11.40
1074	Jones Fife×Red Winter	11,328	11.04
678	Red Winter	11,438	12.25
1152	Turkey	11,444	11.80
1073	Bart Gross-körniger	11,489	11.92
676	Red Winter	11,554	11.42
1082–1	Turkey	11,567	12.48
1110	.....do	11,702	11.80
774	An Australian hybrid	11,712	11.83
1018	Mareuil	11,877	12.00
1111	Turkey	11,912	12.00
936	Bucanera	12,429	10.60
1106	Banat	11,994	11.35
1036	Romanella	12,442	10.76
889	Red Summer Emmer	12,487	11.36
1161	U. S. Cereal Investigation No. 1787	12,753	11.60
1080	U. S. Cereal Investigation No. 1543	12,873	13.20
1066	Macaroni	12,905	12.76
1112	Turkey	12,946	11.56
	Average	9,115	11.73

The correlation between hardness (subject) and protein (relative) is shown in Table II. Table III summarizes the chief constants connected with the calculations.

It is apparent that the correlation between hardness and protein is practically zero. Since this is contrary to common opinion, data from

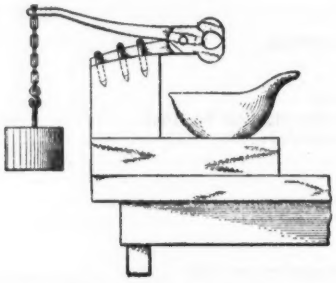

FIG. 1.—Machine used at the California Agricultural Experiment Station for determining the hardness of grain (*4*).

the California Agricultural Experiment Station and from the Kentucky Agricultural Experiment Station—the only existing data aside from those obtained in the present investigations—were examined.

The method used at the California Agricultural Experiment Station for testing the hardness of wheat is that devised and described by Shaw and Gaumnitz (*4*). The apparatus consists simply of a pair of ordinary pliers, one arm of which is stapled to a wooden block (fig. 1).

TABLE II.—*Correlation between hardness of grain (subject) and protein content (relative) in 94 pure strains of Kansas-grown wheats*[a]

			10.00 to 10.49	10.50 to 10.99	11.00 to 11.49	11.50 to 11.99	12.00 to 12.49	12.50 to 12.99	13.00 to 13.49	13.50 to 13.99	14.00 to 14.49	14.50 to 14.99	15.00 to 15.49	15.50 to 15.99	
			10.245	10.745	11.245	11.745	12.245	12.745	13.245	13.745	14.245	14.745	15.245	15.745	
			−1.485	−0.985	−0.485	−0.015	+0.515	+1.015	+1.515	+2.015	+2.515	+3.015	+3.515	+4.015	
3.000	3.499	−5.616	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	1
3.999															
4.000	4.499	−4.616	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	1
4.999															
5.000	5.499	−3.616	1	.....	1	1	.....	.....	.....	.....	.....	.....	.....	1	4
5.999															
6.000	6.499	−2.616	2	6	2	1	5	1	1	.....	.....	.....	.....	.....	18
6.999															
7.000	7.499	−1.616	1	1	1	1	2	1	.....	.....	.....	.....	.....	.....	7
7.999															
8.000	8.499	− .616	.....	.....	.....	1	6	1	.....	1	.....	.....	.....	.....	9
8.999															
9.000	9.499	+ .384	.....	2	4	5	1	3	.....	.....	.....	.....	.....	.....	15
9.999															
10.000	10.499	+1.384	.....	3	5	5	2	2	1	.....	.....	.....	.....	.....	18
10.999															
11.000	11.499	+2.384	.....	1	5	4	4	.....	.....	.....	.....	.....	.....	.....	14
11.999															
12.000	12.499	+3.384	.....	2	1	2	.....	1	1	.....	.....	.....	.....	.....	7
12.999															
			4	15	19	21	21	9	3	1	.....	.....	.....	1	94

[a] First three columns at left denote, respectively, classes of crushing points in kilos, mid points of classes, and deviations from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

TABLE III.—*Summary of crushing points and protein content of 94 pure strains of Kansas-grown wheat*

Mean.		Standard deviation.		Correlation.
Crushing point.	Protein content.	Crushing point.	Protein content.	Coefficient (r).
Gm.	*Per cent.*	*Gm.*	*Per cent.*	
9, 115	11. 73	2, 219. 38	0. 8318	0. 0214
±154. 4	±0. 0015	±109. 17	±0. 0409	±0. 003

The wheat grain is placed between the jaws of the pliers, and weights sufficient to cut the kernel in two are suspended from the extremity of the upper, or fore arm, of the pliers. The writers (*4*) state:

A number of more complicated arrangements were tried, but none seemed to give more uniform results than this simple contrivance. . . . The test was made by counting out 500 kernels, which were crushed by opening the jaws of the pliers just wide enough to insert the grain between them and then allowing the weight to settle gently. The grains remaining unbroken by each weight were set aside and counted. . . . Five different weights were employed, viz, 0.75, 1.00, 1.25, 1.50, and 1.75 pounds.

The breaking point determined in this manner is necessarily very different from the crushing point, as determined in the writer's experiment. This will be apparent by reference to the differences in the methods as described above. In order to correlate the breaking weights and protein content, the former were reduced to grams and the number of broken grains—that is, 100 less the number remaining unbroken—in each case were grouped under the separate weights. Then the sum of the products of this number—that is, the number of grains breaking under each separate weight—multiplied by the breaking weight and divided by the total number of grains crushed, gives an average breaking point in grams that rests upon a common basis with the writer's experiments, except for the differences due to the machine and the method used. The results, expressed as the mean breaking point in grams, are given in Table IV. The protein content is also given for comparison.

TABLE IV.—*Relation of mean breaking point to protein content of California-grown wheats*

WASHINGTON BLUESTEM

Laboratory No.	Number of grains broken by weight of—					Mean breaking point.	Average protein content.
	350.2 gm.	453.6 gm.	567 gm.	680.4 gm.	793.8 gm.		
						Gm.	*Per cent.*
4	0	53	80	0	0	461	8.98
206	5	23	46	0	0	517	8.85
16	0	57	72	0	0	517	10.94
5	0	57	78	0	0	519	13.71
15	0	46	83	0	0	527	10.91
9	0	17	52	0	0	539	9.05
14	0	13	69	0	0	549	12.79
10a	13	29	44	70	7	585	8.76

TABLE IV.—*Relation of mean breaking point to protein content of California-grown wheats*—Continued

WASHINGTOM BLUESTEM—continued

Laboratory No.	Number of grains broken by weight of—					Mean breaking point.	Average protien content.
	350.2 gm.	453.6 gm.	567 gm.	680.4 gm.	793.8 gm.		
						Gm.	*Per cent.*
24	7	32	59	80	0	589	11.51
25	1	46	68	92	0	591	13.87
17	0	28	52	83	0	605	11.91
386	1	23	57	88	0	609	9.53
158	0	11	34	64	0	622	9.27
205	28	37	49	81	93	637	7.18
300	1	9	32	82	0	642	8.95
267	5	26	63	88	94	666	7.36
152	6	16	36	60	95	686	10.26
272	0	14	37	50	78	689	7.15
299	6	9	35	76	89	690	8.08
268	0	12	46	84	98	694	7.29
121	0	5	19	47	87	722	9.01
472	0	1	21	40	85	728	12.26
303	0	0	16	42	75	731	9.31
606	1	2	6	6	40	736	10.30
64	0	0	4	18	41	747	10.14
118	0	0	9	25	58	990	10.68
Average						638	9.93

AUSTRALIAN

78	0	5	42	89	0	156	11.09
309	5	8	20	37	81	271	9.83
62	0	19	53	80	0	290	11.43
288	0	4	14	44	73	294	9.63
23	61	39	11	0	0	408	12.06
141	5	50	74	0	0	514	9.90
138	8	50	77	0	0	512	10.86
134	5	46	77	0	0	518	9.90
310	0	34	82	0	0	533	8.25
184	3	20	78	0	0	538	11.07
319	0	16	57	0	0	542	8.55
66	0	0	7	25	72	560	9.86
129	0	17	50	93	0	621	9.18
127	0	9	63	96	0	625	8.27
322	0	0	7	69	0	670	8.54
275	2	21	55	87	94	676	12.56
77	0	5	42	89	0	637	10.53
452	0	1	16	23	65	688	9.43
326	4	13	28	76	86	692	10.48
232	5	14	23	72	97	697	6.92
311	0	2	9	66	0	700	8.36
69	0	5	39	78	90	702	13.36
231	0	10	12	40	57	704	7.72
67	0	2	12	39	46	715	11.41
453	0	3	15	19	53	721	10.10
22	0	1	15	54	75	725	12.52
28	0	1	15	54	75	726	12.35
26	0	1	10	16	39	727	13.42
312	0	1	8	78	88	731	7.97
73	0	0	10	39	76	740	9.83
68	0	0	10	44	84	741	11.02
605	0	0	0	10	47	773	10.83
Average						598	12.32

TABLE IV.—*Relation of mean breaking point to protein content of California-grown wheats*—Continued

LITTLE CLUB

Laboratory No.	Number of grains broken by weight of—					Mean breaking point.	Average protein content.
	350.2 gm.	453.6 gm.	568 gm.	680.4 gm.	793.8 gm.		
						Gm.	*Per cent.*
50a	77	0	0	0	0	350	8.97
51a	53	79	91	0	0	475	8.40
51a	53	79	91	0	0	475	9.21
317	5	63	82	0	0	512	8.15
11	0	57	69	0	0	516	11.35
8	0	37	61	0	0	524	7.83
277	0	40	71	0	0	526	9.05
144	1	34	79	0	0	531	8.58
49	46	54	86	92	0	554	9.19
21	6	65	73	75	0	566	13.39
305	22	34	59	80	0	569	8.61
143	2	38	70	98	0	597	9.03
315	3	30	88	98	0	599	8.18
139	5	24	66	94	0	603	9.26
290	0	23	52	75	0	606	10.39
55	4	18	59	87	0	608	8.46
130	0	20	55	90	0	615	9.08
124	2	19	45	90	0	616	9.08
128	0	16	64	93	0	617	8.18
28a	6	17	48	87	0	622	9.02
60	28	57	76	90	97	624	8.77
6	0	8	33	87	0	681	13.43
56	1	12	53	83	95	687	10.13
57	1	15	46	75	95	688	8.41
161	0	8	43	80	94	698	8.08
53	0	7	42	77	93	700	8.76
59	0	8	36	71	96	704	8.34
27	2	8	28	29	93	711	13.53
80	0	4	36	70	97	718	10.38
58	0	3	37	77	99	733	8.17
Average						601	9.38

SONORA

137	3	35	76	0	0	526	10.31
257	6	32	58	83	0	592	12.84
140	0	30	71	96	0	605	12.20
249	4	9	25	25	67	691	12.05
167	2	8	29	65	93	705	14.60
63	0	7	25	74	91	710	12.16
74	0	3	13	45	75	727	11.84
76	0	0	6	34	68	745	11.38
245	0	0	7	23	66	750	11.76
33	0	4	13	27	48	775	12.88
481	0	0	0	1	20	788	10.01
Average						692	12.00

TABLE IV.—*Relation of mean breaking point to protein content of California-grown wheats*—Continued.

PROPO

Laboratory No.	Number of grains broken by weight of—					Mean breaking point.	Average protein content.
	350.2 gm.	453.6 gm.	567 gm.	680.4 gm.	793.8 gm.		
						Gm.	*Per cent.*
45	0	24	45	60	83	631	12.00
79	0	6	48	86	0	631	11.90
47	0	8	22	66	93	713	11.90
46	0	0	11	47	73	734	11.96
Average						677	11.94

The data presented in Table IV are summarized in Table V. The constants involved in the calculation of the correlation coefficient are given in Table VI, and the correlation in Table VII.

TABLE V.—*Summary of relation of mean breaking point and protein content of California-grown wheat*

Breaking point.	Protein content.	Breaking point.	Protein content.	Breaking point.	Protein content.	Breaking point.	Protein content.
Gm.	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*
350	8.97	569	8.61	637	10.53	714	8.76
419	12.06	584	8.03	642	8.95	715	11.41
461	8.98	585	8.76	660	10.48	718	10.38
475	8.40	589	11.51	666	7.36	721	10.10
475	9.21	591	13.87	667	10.83	722	9.01
492	10.86	597	9.03	675	9.96	726	9.27
512	8.15	599	8.18	677	7.79	726	13.21
515	13.00	603	9.26	681	13.43	727	9.77
516	11.35	605	11.91	686	10.26	727	10.15
517	8.85	605	12.11	687	10.13	727	13.42
517	10.94	606	10.39	688	8.41	728	12.26
519	13.71	608	8.46	689	7.15	731	7.97
524	7.83	609	9.53	690	8.08	731	9.31
526	7.80	613	11.43	691	9.33	731	9.43
526	9.05	615	9.08	694	7.29	733	8.17
527	10.91	616	9.08	698	8.08	734	10.50
531	8.58	617	8.18	698	9.54	736	10.30
534	8.25	622	9.02	700	8.76	740	9.83
538	11.07	622	9.27	702	13.36	741	11.02
539	9.05	624	8.77	703	9.38	747	10.14
542	8.55	625	9.18	704	8.34	750	8.83
549	9.90	626	8.27	710	14.60	768	8.36
549	12.79	628	11.07	710	15.80	779	8.54
554	9.19	632	11.76	711	7.72	788	10.01
560	9.08	636	6.92	711	13.53	788	9.63
566	13.39	637	7.18	713	8.76	990	10.68

TABLE VI.—*Constants involved in the calculation of correlation coefficient of mean breaking point and protein content of California wheats*

Mean.		Standard deviation.		Correlation coefficient (r).
Crushing point.	Protein content.	Crushing point.	Protein content.	
Gm.	*Per cent.*	*Gm.*	*Per cent.*	
631	9.89	89.90	1.8058	0.0111
±5.9176	±0.1194	±4.1853	±0.0841	±0.061

The results corroborate the Kansas data, with a correlation coefficient of practically zero, and a probable error larger than the correlation coefficient.

In the Kentucky experiments, conducted by Harper and Peters (*2*), the hardness of the kernels was determined by means of a piston with a

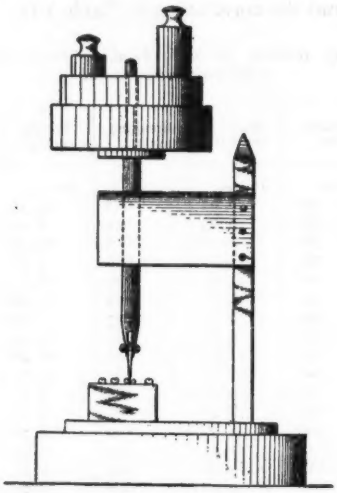

FIG. 2.—Machine used at the Kentucky Agricultural Experiment Station for testing the hardness of wheat, designed by J. N. Harper (2).

cutting edge, which is pressed down upon the kernel by means of weights added directly from above (fig. 2). The kernel is cut in two and is not crushed.

The opinion of the Kentucky investigators regarding the apparatus used is as follows:

> This device for testing the hardness of wheats appears to us to be superior to those that measure the pressure required to crush a kernel.

The data obtained are presented in Table VIII.

TABLE VII.—*Correlation between hardness of grain (subject) and protein content (relative) in 103 varieties of California-grown wheat* [a]

			7.00 to 7.49	7.50 to 7.99	8.00 to 8.49	8.50 to 8.99	9.00 to 9.49	9.50 to 9.99	10.00 to 10.49	10.50 to 10.99	11.00 to 11.49	11.50 to 11.99	12.00 to 12.49	12.50 to 12.99	13.00 to 13.49	13.50 to 13.99	14.00 to 14.49	14.50 to 14.99	15.00 to 15.49	15.50 to 15.99	
			7.245	7.745	8.245	8.745	9.245	9.745	10.245	10.745	11.245	11.745	12.245	12.745	13.245	13.745	14.245	14.745	15.245	15.745	
			−2.625	−2.125	−1.625	−1.125	−0.625	−0.125	+0.575	+0.875	+1.375	+1.875	+2.375	+2.875	+3.375	+3.875	+4.375	+4.875	+5.375	+5.875	
300–399	349	−290.65	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
400–499	449	−190.65	.....	.....	1	1	1	.....	.....	1	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	5
500–599	549	− 90.65	.....	2	4	5	5	1	.....	2	2	1	.....	1	2	2	.....	.....	.....	.....	27
600–699	649	+ 9.35	5	1	6	2	7	3	4	2	2	2	1	.....	1	.....	.....	.....	.....	.....	35
700–799	749	+ 10.935	.....	2	3	5	5	3	6	1	2	.....	1	.....	3	1	.....	1	.....	1	34
800–899	849	+ 20.935	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
900–999	949	+ 30.935	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
1,000–1,099	1,049	+ 40.935	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1,100–1,199	1,149	+ 50.935	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
			5	5	14	14	18	7	10	7	6	3	3	1	6	3	.....	1	.....	1	103

[a] First three columns, at left, denote, respectively, classes of breaking points in grams, mid points of classes, and deviations from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

TABLE VIII.—*Protein content and hardness of Kentucky wheats*

Variety.	Protein content.	Number of kernels in 100, cut under a pressure of 4 pounds (1,818 gm.).	
		Flinty grains.	Starchy grains.
	Per cent.		
Ruby	11.63	40	56
Turkey Red	11.85	68	96
Gold Coin	12.33	94	98
Canadian hybrid	12.50	78	86
Dawson's Golden Chaff	12.63	68	92
Indiana Swamp	12.77	56	60
Harvest Queen	13.01	96	98
Hungarian	13.12	80	96
Lancaster Red	13.26	76	96
Pearl's Prolific	13.91	68	86
Fulcaster	14.01	46	68
Kansas Mortgage Lifter	14.04	56	84
Harvest King	14.06	70	90
Fultz	14.25	75	98
Jersey Fultz	14.66	80	94
Extra Early Oakley	14.68	55	66
Beechwood hybrid	14.95	76	78
Improved Rice	15.17	66	92
Pootung	16.55	50	70

The data from the Kentucky Agricultural Experiment Station are not presented in the same manner as those from the Kansas and the California Stations and can not be considered in exactly the same way. Instead of the number of kernels crushed under different weights, the percentages of kernels crushed under the same weight (4 pounds, or 1,818 gm.) are given. The kernels are divided into two classes, flinty and starchy, and the percentage number of kernels out of 100, cut with the 4-pound weight, is given for each class.

TABLE IX.—*Constants involved in the calculation of the correlation coefficient of mean breaking point and protein content of Kentucky wheat*

Mean.		Standard deviation.		Correlation coefficient.
Percentage of kernels cut.	Protein content.	Percentage of kernels cut.	Protein content.	
	Per cent.		*Per cent.*	
68.18	13.65	14.58	1.0039	0.0335
±0.2256	±0.1553	±1.5953	±0.1098	±0.1545

Thus, it is not possible to correlate the percentages of protein with a series of different crushing or breaking points as in the other experiments. This difficulty was avoided by calculating the correlation coefficient of the percentages of flinty kernels which were cut under the given weight

with the percentage of protein. It seems that if any correlation exists, those varieties having the lowest percentage of kernels cut—the hardest varieties—should have the highest percentages of protein, and vice versa. The results as calculated by the writer are given in Table IX. The correlation table is given in Table X.

Here also the correlation between the hardness of the wheat kernels and the protein content is not significant.

TABLE X.—*Correlation between hardness of grain (subject) and protein content (relative) in 19 varieties of Kentucky-grown wheat* [a]

			11.00 to 11.99 11.495 −2.155	12.00 to 12.99 12.495 −1.155	13.00 to 13.99 13.495 −0.155	14.00 to 14.99 14.495 +0.845	15.00 to 15.99 15.495 +1.845	16.00 to 16.99 16.495 +2.845	
40 49	44.5	−23.7	1	.....	.....	1	.....	.....	2
50 59	54.5	−13.7	.....	1	.....	2	1	.....	4
60 69	64.5	− 3.7	1	1	1	.....	1	.....	4
70 79	74.5	+ 6.3	.....	1	1	3	.....	.....	5
80 89	84.5	+16.3	.....	.....	1	1	.....	.....	2
90 99	94.5	+26.3	.....	1	1	.....	.....	.....	2
			2	4	4	7	2	.....	19

[a] First three columns at left denote, respectively, classes of percentages of flinty kernels cut under pressure of 4 pounds (1,818 gm.), mid points of classes, and deviations from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

TABLE XI.—*Coefficient of correlation constants*

Number of strains.	Mean.			Standard deviation.			Correlation coefficient.	
	Specific gravity.	Protein content.	Volume.	Specific gravity.	Protein content.	Volume.	Between specific gravity and protein content.	Between volume and protein content.
		Per cent.	*Mm.*		*Per cent.*	*Mm.*		
67	1.32	11.67	.....	0.04	0.754	.....	.....	.....
	±0.003	±0.062		±0.002	±0.062			
68	.....	11.67	20.60	.....	0.73	1.0089	0.0587	0
		±0.059	±0.082		±0.042	±0.057	±0.081	

The correlation coefficient between hardness and protein content proving to be practically zero, a study was then made to determine the correlation between specific gravity and protein content and between kernel volume and protein content of the Kansas pure strains. The correla-

tions are given in Tables XII and XIII. The chief constants in connection with the calculation of the correlation coefficient are presented in Table XI.

TABLE XII.—*Correlation between specific gravity (subject) and protein (relative) in 67 strains of Kansas-grown wheat* [a]

			10.00 to 10.49 10.245 1.425	10.50 to 10.99 10.745 .925	11.00 to 11.49 11.245 .425	11.50 to 11.99 11.745 .075	12.00 to 12.49 12.245 .525	12.50 to 12.99 12.745 1.075	13.00 to 13.49 13.245 1.575	
1.10 1.14	1.12	0.20	.....	.....	.....	.....	.....	.....	.....	...
1.15 1.19	1.17	.15	.....	1	.....	.....	.....	.....	.....	1
1.20 1.24	1.22	.10	.....	.....	.....	.....	.....	.....	.....	...
1.25 1.29	1.27	.05	1	.....	2	3	4	1	1	12
1.30 1.34	1.32	.00	2	10	5	6	8	4	.....	35
1.35 1.39	1.37	.05	.....	2	4	4	5	2	1	18
1.40 1.44	1.42	.10	.....	.....	1	.....	.....	.....	.....	1
			3	13	12	13	17	7	2	67

[a] First three columns at left denote, respectively, classes of specific gravity, mid points of classes, and deviations from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

TABLE XIII.—*Correlation between volume of kernel (subject) and protein (relative) in 68 strains of Kansas-grown wheat* [a]

			10.00 to 10.49 10.245 −1.425	10.50 to 10.99 10.745 −.925	11.00 to 11.49 11.245 −.425	11.50 to 11.99 11.745 +.075	12.00 to 12.49 12.245 +.575	12.50 to 12.99 12.745 +1.075	13.00 to 13.49 13.245 +1.575	
15 19	17	− 3.60	1	5	3	4	8	3	1	25
20 24	22	+ 1.40	2	7	10	7	10	2	.....	38
25 29	27	+ 6.40	.....	.....	1	1	1	.....	1	4
30 34	32	+11.40	.....	.....	1	.....	.....	.....	.....	1
			3	12	15	12	19	5	2	68

[a] First three columns at left denote, respectively, classes of volumes, mid points of classes, and deviation from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

There seems to be no correlation between protein content, on the one hand, and either specific gravity or volume of the grain, on the other, as was found to be true for hardness and protein content.

The absence of correlation between hardness of the grain and percentage of protein is remarkable, in view of the very general belief that the hard wheats are relatively high in protein, and the fact that this belief is supported by strong indirect evidence.

SUMMARY

(1) It has been assumed, and there is some evidence to show, that hardness of wheat is associated with and possibly dependent upon its protein content, the harder wheats being the higher in protein.

(2) In the breeding of hard winter wheat, protein is one of the most important factors next to yield to be considered. This being the case, it is desirable to discover a method of selecting for protein that will dispense with the necessity of making chemical analyses of large numbers of varieties or strains under preliminary trial.

(3) If hardness and protein content are genetically related, then when large numbers are taken the coefficient of correlation should show such relationship. This being the case, the selection of strains for hardness should involve selection for protein also.

(4) Three methods of testing wheat for hardness have been devised: That of the writer at the Kansas Agricultural Experiment Station (*3*); that of Harper and Peters, of the Kentucky Agricultural Experiment Station (*2*); and that of Shaw and Gaumnitz, of the California Agricultural Experiment Station (*4*). Calculating the correlation between the crushing or breaking point of the kernel in grams and the percentage of protein for the data from the three Stations, we have for the Kansas data a correlation coefficient of only 0.02 ± 0.003, for the California data 0.01 ± 0.061, and for the Kentucky data 0.03 ± 0.1545. These consistently negative results from the three sources seem inexplicable in the light of the generally accepted belief that the harder wheats are usually higher in protein.

(5) The correlation coefficient between specific gravity and protein and between volume of the grain and protein were also determined. The correlation coefficient was found to be $0.05 \pm$ in the first instance and zero in the second.

(6) The writer is at a loss thus far to account for the conflict between the generally recognized fact, on the one hand, that the higher protein wheats are the harder wheats, and the entire absence of demonstrable correlation between protein content and hardness by means of the correlation coefficient, on the other.

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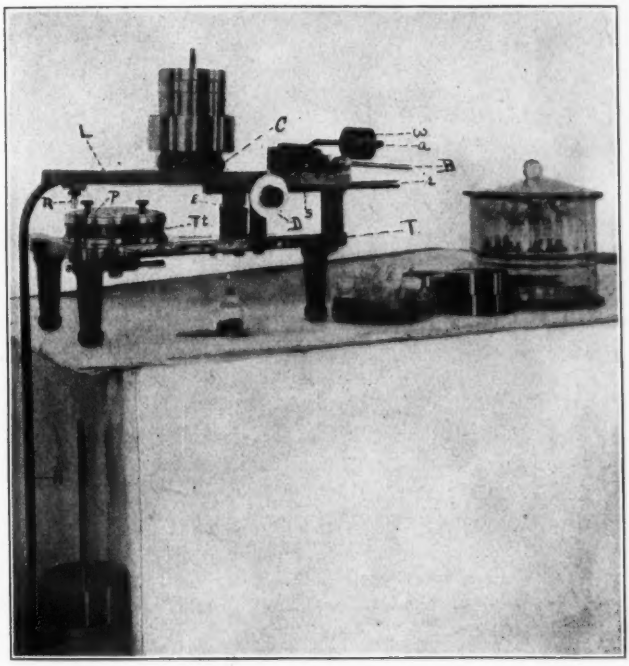
PLATE 100

Machine for determining the hardness of grain in terms of the crushing point, designed by the writer for use at the Kansas Agricultural Experiment Station (*3*). *T*, table; *L*, lever arm graduated to tenths and hundredths; *C*, carrier which runs on lever arm as a track on flanged wheels; *D*, dial which connects with sliding arm, *r*, which in turn moves the carrier *C*; *H*, hanger which rests upon lever *L* by a knife-edge bearing surface. At base of hanger is a support with upright pin upon which perforated weights are hung. *R*, ram or hammer fastened to under side of lever arm; *P*, pans in turntable upon which the grains are laid for crushing; *Tt*, turntable revolved on notched gear by means of spokes underneath; *W*, counterweight adjustable along arm *a*. Weights are brass and are in 1-, 2-, and 5-kgm. pieces, calibrated by the United States Bureau of Standards.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EFFECTS OF SOME CUCURBITA SEEDS ON ANIMAL METABOLISM

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I. INFLUENCE OF PUMPKIN AND SQUASH SEEDS UPON KIDNEY EXCRETION

The plants of the pumpkin family, *Cucurbita pepo* L., *C. moschata* Duchesne, and *C. maxima* Duchesne, are natives of the American continent, but the fruits of both pumpkin and squash are used to a greater extent in some European countries than in the United States. For instance, in Ukraine (Little Russia) the pulp is fed to the farm live stock and some seeds are given to the farm animals when the entire fruit is fed, but a large quantity of the pumpkin and squash seeds is consumed as a dainty by the people.

PURPOSE AND PLAN OF EXPERIMENT

The belief is held by many farmers that the seeds of pumpkin and squash have injurious effects upon the animal when fed in the amounts found in the entire fruit, but no careful experiments are on record to disprove the belief that unless the pumpkin and squash seeds are fed in excess they are not harmful. The purpose of this experiment is to throw some light on this question.

Since the main objection to feeding whole pumpkins lies in the opinion that the seeds cause abnormal urination, the writer of this paper experimented on himself by including in the daily diet given amounts of pumpkin and squash seeds freed from the seed coat and analyzing the daily volumes of the kidney excretion.

The experiment was carried on in two distinct periods, each period consisting of three consecutive days. The first period included March 17, 18, and 19. The second period [2] covered March 24, 25, and 26. During the first period a basal diet was followed, while during the second period an addition of given quantities of pumpkin and squash seeds without the hulls was used.

Although data of all the three days for each period are given in the tables, the results of only the last two days of each period were taken for comparison. The reason was that the results of the first day of each period might represent partial metabolic products from the day previous to the experiment.

[1] I wish to express my thanks to Dr. A. R. Moore, head of the Biological Dept. of Rutgers College, for helpful suggestions in this work and to Prof. H. R. Lewis, Poultry Husbandman at the New Jersey Agricultural Experiment Station, Prof. Frank G. Helyar, Prof. W. Ch. Skelly, and Mr. J. Thompson for assistance with the experiments.

[2] During March 20, 21, 22, and 23 small quantities of pumpkin and squash seeds were included in the diet to accustom the digestive system to the material to be used in the second period of the experiment.

BASAL DIET (PERIOD I)

The basal diet consisted of the following meals for each day: Morning, one cup of coffee, three wheat biscuits, one bowl of oatmeal and milk, and some oleomargarin; noon, two fried eggs, one glass of water, a quantity of rice pudding, three slices of bread, one cup of coffee, and some oleomargarin; evening, 1 pint of milk and 4 wheat biscuits. Since the meals were served at the same restaurant during both periods, the quantities of oatmeal, rice pudding, and bread and butter (oleomargarin) were approximately the same.

The bladder was emptied about 7 a. m. and the urine discarded. All the urine from that hour up to and including that passed the next day at 7 a. m. was saved, thoroughly mixed, preserved with toluene, and kept in 1-liter Erlenmeyer flasks with rubber stoppers. Thus, each composite sample represented the mixed excretion for 24 hours. The preservative, C. P. toluene ($C_6H_5.CH_3$), was used at the rate of 5 cc. per liter of content.

As soon as a 24-hour sample was secured it was immediately analyzed for the determination of volume, specific gravity, titratable acidity (using *N/10* sodium hydroxid), and ammonia content, employing the formol titration method of Malfatti (*3, p. 502*).[1] The total solids of a sample were computed by means of Long's coefficient (*3, p. 483*). During the intermediary period, including March 21, 22, 23, and 24, the estimation of creatinin by Folin's colorimetric method (*3, p. 506*) and the determination of total nitrogen by the Folin-Farmer microchemical method (*3, p. 485*) was accomplished for the three composite samples of the first period.

TABLE I.—*Analyses of composite samples of urine from basal diet period*

Date.	Volume.	Temperature.	Specific gravity.	Total solids.	Acidity.[a]	Ammonium hydrates (NH_3).	Creatinin.	Total nitrogen.
	Cc.	°*C.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*
Mar. 17.....	1,170	25	1.024	73.008	383.70	1.02314	1.404	8.353
18.....	740	25	1.031	59.64	441.04	1.03348	1.361	6.364
19.....	650	25	1.033	55.77	451.88	0.9447	.993	7.462

[a] Expressed in cubic centimeters of *N/10* sodium hydroxid required to titrate 100 cc. of sample

EXPERIMENTAL DIET (PERIOD II)

The pumpkin and squash seeds used in the experimental diet were obtained from a canning factory where pumpkin and squash fruits are packed. To increase the palatability and to remove the effects of possible unsanitary handling of the pumpkin and squash seeds they were spread in a thin layer on a tray and placed in a dry heat oven where the material was kept at a temperature of about 150° C. until a slight browning of the seed coats was noticed, indicating that the heating had been sufficient to destroy all bacteria present and to roast the

[1] Reference is made by number (italic) to "Literature cited," pp. 358-539.

kernels. This operation also lightened the process of removing the hulls from the seeds. The weight of the seed consumed per day was taken by subtracting the weight of the hulls from the amount of seeds used.

The experimental diet consisted of practically the same meals as the basal diet, except for the addition of pumkpin and squash seeds to the evening meals. On March 24, 91 gm. of pumpkin and squash seeds were eaten;[1] on March 25, 31 gm., and on March 26, 38 gm. The meal hours and study hours during this period II were exactly the same as in period I, and all other controllable conditions were kept as uniform as possible with those of period I.

The same methods of sampling and analyzing employed in period I were used in period II.

TABLE II.—*Analyses of composite samples of urine from experimental diet period*

Date.	Volume.	Temperature.	Specific gravity.	Total solids.	Acidity.[a]	Ammonium hydrate (NH_3).	Creatinin.	Total nitrogen.
	Cc.	°*C.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*
Mar. 24[b]	1,195	25	1.022	68.35	415.8	1.10274	0.997	9.191
25	570	25	1.037	54.83	465.1	1.01688	.721	4.651
26	545	25	1.038	55.26	525.7	1.1846	.735	7.913

[a] Expressed in cubic centimeters of *N*/10 sodium hydroxid necessary to titrate 100 cc of sample.

[b] The slightly greater volume of urine excreted during the first day (Mar. 24) of period II as compared with that of March 17 of period I is ascribed to the abnormal condition resulting from the excess of pumpkin and squash seeds ingested at the evening meal of that day.

The estimation of the total phosphoric acid (P_2O_5) and total sulphuric acid (SO_3) of the samples of both periods was done during the latter part of April. The uranium acetate method (*3, p. 552*) was employed for the determination of P_2O_5 and Folin's method (*3, p. 546*) was used for the estimation of SO_3. Table III shows the results for both periods.

TABLE III.—*Determination of P_2O_5 and SO_3*

Date of sample.	P_2O_5.	SO_3.	Mean of the last two samples.	
			P_2O_5.	SO_3.
Period I:	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Mar. 17	3.229	1.876	.....	.....
18	2.434	1.888	2.484	1.870
19	2.535	1.853		
Period II:				
Mar. 24	2.909	1.944	.....	.....
25	2.513	2.321	2.578	2.300
26	2.643	2.280		

[1] About eight hours after the evening meal on March 24 acute stomach pain accompanied by eructation into the mouth of a very acid solution took place. To relieve this condition the back of the pharynx was irritated with the finger, causing vomiting.

The content of total sulphates was almost 123 per cent in the hypothetical mean sample of period II as compared with the same of period I.

COMPOSITION OF SEEDS

The composition of the pumpkin and squash seeds (less the hulls) which were used during period II of this experiment is given in Table IV.

TABLE IV.—*Commercial analysis of pumpkin and squash seeds (less hulls)*

Ingredient.	Per cent.	Ingredient.	Per cent.
Protein	33.64	Fiber	1.42
Fat	53.48	Water	2.77

The low water content was due to the fact that the kernels were roasted. The high percentage of fat and protein keeps the same relation to the water content in raw seeds.

Comparing the results shown in Tables I and II, it is noted that not only the volume of kidney excretion during the experimental diet period is considerably less than that during the basal diet period but also the amount of total solids is about 5 per cent less in the former as compared with the total solids calculated for the excretion during the basal diet period. The marked differences are well illustrated in the supplementary Tables V and VI.

TABLE V.—*Comparison of volume of urine excreted during periods I and II*

Date of sample.	Volume.	Hypothetical mean.	Percentage of volume of period II as compared with that of of period I taken as 100.
Period I:	*Cc.*	*Cc.*	
Mar. 18	740	695	.....
19	650		
Period II:			
Mar. 25	570	557.5	80.2
26	545		

Since a slight increase or decrease in the output of urine, so far as volume is concerned, is not of great significance, the determination of the creatinin and total nitrogen, as found in the samples of periods I and II, was made. The results and comparison of both periods relating to the output of creatinin and total nitrogen is shown in Tables VII and VIII.

The data presented in these tables clearly indicate not only that the volume of urine and the amounts of total solids excreted during the experimental diet period were considerably less than during the basal

diet period but also that the percentages of total creatinin and total nitrogen in the urine during the experimental diet period was significantly less than during the basal diet period.

TABLE VI.—*Comparison of total solids of urine excreted during periods I and II*

Date of sample.	Total solids.	Hypothetical mean.	Percentage of total solids of period II compared with that of period I taken as 100.
Period I:	*Gm.*	*Gm.*	
Mar. 18	59.64	57.70	.....
19	55.77		
Period II:			
Mar. 25	54.83	55.04	95.3
26	55.26		

TABLE VII.—*Comparison of creatinin in urine of periods I and II*

Date of sample.	Creatinin.	Hypothetical mean.	Percentage of creatinin of period I compared with that of period II taken as 100.
Period I:	*Gm.*	*Gm.*	
Mar. 18	1.361	1.177	.....
19	.993		
Period II:			
Mar. 25	.721	.728	61.9
26	.735		

TABLE VIII.—*Comparison of total nitrogen in urine of periods I and II*

Date of sample.	Total nitrogen.	Hypothetical mean.	Percentage of total nitrogen of period I compared with that of period II taken as 100.
Period I:	*Gm.*	*Gm.*	
Mar. 18	6.364	6.913	.....
19	7.462		
Period II:			
Mar. 25	4.651	6.282	90.8
16	7.913		

Although the results of a single experiment of this nature do not suffice to disprove the belief prevailing among stockmen that pumpkin and squash seeds when fed to live stock exhibit a very marked diuretic

action, nevertheless the data presented above do illustrate a case which seems to reverse the general belief. The experimental diet contained a greater amount of protein and fat than the basal diet because of the addition of the pumpkin and squash seeds to the former. It would be expected that this increase of protein and fat constituents would tend to increase the proteid and nonproteid nitrogen content of the urinary excretion of the experimental period, but the results obtained show the contrary to be the case.

SUMMARY OF PART I

1. Contrary to the belief prevalent among stockmen, ingested pumpkin and squash seeds inhibited kidney secretion.

2. Not only is the volume of urine smaller when pumpkin and squash seeds are ingested but the total solids of the urine are diminished.

3. When pumpkin and squash seeds are included in the ration the acidity of the urine tends to rise.

4. An excess of pumpkin and squash seeds in a given ration causes digestive disorders.

5. The results of lower total nitrogen and creatinin in the urine when pumpkin and squash seeds are ingested is of a chemico-pharmacognosic significance.

II. EFFECT OF PUMPKIN SEEDS ON THE METABOLISM OF YOUNG PIGS

The data reported in part I of this paper prompted the investigation of the effect of pumpkin seeds on the metabolism of young pigs. The results of such investigation may lead to an efficient utilization of the by-products of the canning factories where pumpkin and squashes are packed.

In the experiments reported in the literature on feeding pumpkins to pigs (*1*, *2*) and to cattle (*4*, *5*, *6*) the only object was to determine the comparative economical feeding value of the fruits of the pumpkin family. Although it is stated in some of the scattered experimental work on feeding pumpkins to live stock that the claim made regarding the injurious effect of seeds is without foundation (*6*), no experimental data are available to support these statements.

The object of this experiment was to secure satisfactory data bearing directly on the influence of pumpkin seeds fed to young pigs.

EXPERIMENTAL WORK

On October 26, 1920, 18 pigs which were farrowed during August, 1920, were selected for this work. The age of these animals ranged between 2½ and 3 months. Pure-bred Duroc-Jerseys were used. The experiment was conducted under dry-lot conditions at the central hog barn of the New Jersey Agricultural Experiment Station. Until the animals were chosen for this experiment they were running with their

dams in one of the main hog lots where field corn was grown. They were weaned about three weeks prior to the experiment.[1] The males were castrated about two weeks previous to experimentation. The 18 pigs selected were divided into three lots, each consisting of 6 pigs, 3 barrows and 3 sows. The pens in which the pigs were confined were similar in every respect. Previous to experimental feeding the pigs received standard wheat middlings as a thin, wet mash, and whole corn, hand-fed.

On October 26 the animals were ear-tagged for identification and dipped in about a 3 per cent creolin solution against vermin. Four days were allowed for the animals to adapt themselves to the new conditions.

On October 30 their initial weights were taken (Table IX). Each self-feeder of each pen was filled with known amounts of yellow dent corn kernels, digester tankage, and standard wheat middlings. The "cannery" seeds used in this experiment were purchased from a canning factory where pumkpins and squashes were packed. The pumpkins fed to the animals were the ordinary sugar pumpkin (*Cucurbita pepo*) chopped up fine (about 1 cc. in size). On November 1 the animals were dewormed by administering internally through the mouth 32 drops of worm-seed oil to 1 fluid ounce of castor oil to each pig.

TABLE IX.—*Initial weights of pigs*

Lot No.	Tag No.	Sex.	Weight.
			Pounds.
I	626	Female	30.0
	627	Barrow	31.0
	628	Female	31.0
	629	do	28.0
	630	Barrow	25.5
	631	do	35.0
Total			180.5
II	632	Barrow	29.0
	633	Female	32.0
	634	do	39.0
	635	Barrow	27.0
	636	Female	32.5
	637	Barrow	42.5
Total			202.0
III	638	Barrow	42.0
	639	Female	26.0
	640	do	24.5
	641	Barrow	35.0
	642	Female	34.5
	643	Barrow	27.0
Total			189.0

[1] The caked condition of the udder of some of the dams was responsible for the rather late weaning time.

To analyze the pumpkin, a thoroughly mixed sample of several finely chopped pumpkins containing the seeds was taken, as well as a thoroughly mixed sample of several finely chopped pumpkins without the seeds. These samples were weighed and registered, then placed in a drying oven where they were kept for two days. The moisture content of the pumpkins without the seeds was 95.82 per cent; that of pumpkins with the seeds was 91.99 per cent; and that of the seeds was 56.60 per cent. The proportion of seed to entire fruit was obtained by weighing eight consecutive daily feedings of pumpkins before and after their seeds were removed. A list of the determinations follows.

Date.	Entire pumpkin.	Seeds.
	Pounds.	*Pounds.*
Nov. 13	13.5	1.5
14	12.5	1.2
15	13.5	1.5
16	14.0	1.2
17	12.5	1.5
18	15.0	1.3
19	16.0	2.2
20	10.0	1.0
Total	107.0	[a] 11.4

[a] 10.65 per cent of weight of entire pumpkin.

The Table X gives the composition of the feed used. The determinations for crude protein, crude fat, crude fiber, and moisture were made by Charles S. Cathcart, State chemist; the analyses for ash and nitrogen-free extract were made by the writer.

TABLE X.—*Composition of feed*

Feed.	Water.	Ash.	Crude protein.	Nitrogen-free extract.	Crude fiber.	Crude fat.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Digester tankage	10.46	17.84	56.56	5.32	1.47	8.35
Middlings	10.07	4.62	16.75	54.91	8.64	5.01
Dent corn	10.96	.83	9.44	73.12	1.23	4.42
"Cannery" seeds	6.45	3.51	29.06	9.31	16.17	35.50
Pumpkin pulp (dry)[a]		8.92	8.77	64.25	15.17	2.89
Pumpkin seed (dry)[a]		6.47	33.38	11.09	10.66	38.40

[a] Calculations were made on water-free basis.

The duration of the experiment was 62 days, beginning October 30 and closing December 31. The experiment was arranged and conducted in four periods.

Period I. Entire pumpkin tested against pumpkin less the seeds.

Period II. Entire pumpkin and ground "cannery" seeds tested against entire pumpkin.

Period III. Entire pumpkin and ground "cannery" seeds tested for their value.

Period IV. Ground "cannery" seeds in a dry mash tested for their value.

PERIOD I.—This period began October 30 at noon and closed November 27 at noon. The object during this period was to compare results obtained by feeding pumpkins from which the seeds have been removed with the results obtained by feeding the entire pumpkin. Lot I received during the first two weeks, besides the feed in the self-feeder ad libitum, finely chopped pumpkin containing 10.65 per cent of seeds. During the last two weeks of this period the percentage of seeds in the pumpkin was increased to 21.30 by adding loose seeds. Lot II received the feed found in the self-feeder and served as a control. Lot III received, besides the feed in the self-feeder ad libitum, chopped pumpkin from which the seeds were removed.

The results of this period are given in Tables XI to XIII.

TABLE XI.—*Feed consumed and effect on weight in period I*

Lot No.	Date.	Nutrients per 100 pounds live weight.				Gain or loss per hundredweight.
		Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	Oct. 30 to Nov. 6	6.267	2.189	25.151	1.243	5.52
	Nov. 6 to Nov. 13	6.510	2.458	25.057	1.605	8.66
	Nov. 13 to Nov. 20	6.838	3.119	24.977	1.527	9.05
	Nov. 20 to Nov. 27	6.621	3.026	23.624	1.410	10.25
	Average per week	6.559	2.698	24.702	1.446	8.37
I_1	Oct. 30 to Nov. 6	6.706	2.108	26.768	1.122	5.19
	Nov. 6 to Nov. 13	5.599	1.957	26.752	1.312	5.88
	Nov. 13 to Nov. 20	5.524	1.995	28.211	1.018	9.55
	Nov. 20 to Nov. 27	5.744	1.958	26.683	.884	10.64
	Average per week	5.893	2.004	27.103	1.084	7.81
III	Oct. 30 to Nov. 6	6.608	2.063	26.374	1.279	10.31
	Nov. 6 to Nov. 13	4.617	1.391	17.250	1.035	—.23
	Nov. 13 to Nov. 20	5.407	1.748	23.118	1.193	6.25
	Nov. 20 to Nov. 27	5.659	1.710	21.262	1.239	6.67
	Average per week	5.572	1.728	22.001	1.186	7.66

Since a comparison of results obtained can be better made by figuring out the amount of nutrients required per pound of gain as well as the actual quantities of the different feeds consumed during a given period, these are given in Tables XII and XIII.

TABLE XII.—*Feed consumed during period I*

Lot No.	Date.	Corn per hundredweight.	Middlings per hundredweight.	Tankage per hundredweight.	Pumpkin per hundredweight.		Mean weekly temperature.[a]	
					Pulp.	Seeds.	Maximum.	Minimum.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*° F.*	*° F.*
I	Oct. 30 to Nov. 6	27. 53	7. 97	3. 65	14. 06	1. 67	63	40
	Nov. 6 to Nov. 13	23. 72	12. 07	2. 99	28. 61	3. 41	56	39
	Nov. 13 to Nov. 20	27. 04	7. 00	3. 38	30. 41	8. 33	45	31
	Nov. 20 to Nov. 27	26. 13	5. 73	3. 52	30. 54	8. 26	48	38
	Average per week	26. 10	8. 19	3. 38	25. 90	5. 42	.....	.....
II	Oct. 30 to Nov. 6	30. 34	7. 92	4. 45	.....	.....	63	40
	Nov. 6 to Nov. 13	28. 32	10. 82	1. 97	.....	.....	56	39
	Nov. 13 to Nov. 20	33. 42	6. 66	2. 22	.....	.....	45	31
	Nov. 20 to Nov. 27	32. 45	5. 07	3. 24	.....	.....	48	38
	Average per week	31. 14	7. 61	2. 97	.....	.....	.....	.....
III	Oct. 30 to Nov. 6	28. 83	7. 72	4. 39	30. 58	.....	63	40
	Nov. 6 to Nov. 13	17. 17	6. 71	3. 11	31. 41	.....	56	39
	Nov. 13 to Nov. 20	24. 75	6. 97	3. 12	38. 22	.....	45	31
	Nov. 20 to Nov. 27	21. 49	7. 91	3. 84	37. 10	.....	48	38
	Average per week	23. 06	7. 32	3. 61	34. 32	.....	.....	.....

[a] The temperature data were secured from the Cooperative Observers' Meteorological Records of New Jersey Agricultural Experiment Station, New Brunswick, N. J.

TABLE XIII.—*Nutrients required per pound of gain*

Lot No.	Date.	Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
I	Oct. 30 to Nov. 6	1. 135	0. 392	4. 556	0. 225
	Nov 6 to Nov. 13	. 751	. 296	2. 893	. 185
	Nov. 13 to Nov. 20	. 755	. 344	2. 759	. 168
	Nov. 20 to Nov. 27	. 645	. 295	2. 304	. 137
	Average per week	. 821	. 331	3. 128	. 178
II	Oct. 30 to Nov. 6	1. 292	. 406	5. 157	. 216
	Nov. 6 to Nov. 13	. 952	. 332	4. 549	. 223
	Nov. 13 to Nov. 20	. 578	. 208	2. 954	. 106
	Nov. 20 to Nov. 27	. 539	. 184	2. 507	. 083
	Average per week	. 840	. 282	3. 791	. 157
III	Oct. 30 to Nov. 6	. 640	. 200	2. 558	. 124
	Nov. 6 to Nov. 13	.....	.....	.....	.....
	Nov. 13 to Nov. 20	. 865	. 279	3. 698	. 198
	Nov. 20 to Nov. 27	. 848	. 256	3. 187	. 185
	Average per week	. 784	. 245	3. 147	. 126

The feeding of the pumpkin was done regularly three times a day, by hand. Considering the young age of the experimental animals it was expected that they would consume 5 pounds of pumpkin per 100 pounds live weight per day, but the first week revealed that lot I would clean up the seeds at first and not go very readily after the pulp, while lot III would consume a little over 4 pounds per day per 100 pounds live weight. However, beginning with the second week the total weight of the entire pumpkin consumed by lot I would be equal to the total weight of pumpkin less the seeds consumed by lot III. During the first three weeks of this period considerable quantities of the whole seeds were found in the feces. Later on, however, this condition became rare. Toward the end of period I it became evident that pig 637 of lot II was gaining out of proportion as compared with the rest of the lot; on the other hand, pig 639 was a runt, being at a standstill. Pig 642 of lot III began to develop a rupture at the umbilicus. Before beginning period II these three pigs were discarded. To balance the numbers in the pens, pig 626 of lot I was transferred to pen No. 3, leaving five pigs per pen. Every effort was made to keep the lots as nearly uniform as possible.

The pigs were weighed weekly. The feeds of the self-feeders were weighed at the close of each week, and the weekly consumption of the feeding stuffs was thus obtained. For the computation of the consumption and the gain or loss during the week per 100 pounds live weight the initial weight of each lot of each week served as the basis for calculation.

During the second week of period I lot III consumed relatively less corn than each of the first two lots; hence, the gain of lot III was affected. With the exception of pig 630, lot I gave more favorable results throughout the entire period than either of the other two lots.

PERIOD II.—This period began November 27 at noon and closed December 11 at noon. The object during this period was to compare results obtained by feeding known quantities of ground "cannery" seeds mixed with the chopped pumpkin (containing 10.65 per cent seeds) with the results obtained by feeding the chopped pumpkin (containing 10.65 per cent seeds) alone. Lot I received the mixture of pumpkin products, fed by hand, besides the feed contained in the self-feeder ad libitum. Lot II was on the self-feeder only, serving as a control. Lot III received chopped pumpkin (containing 10.65 per cent seeds) fed by hand, besides the foods of the self-feeder ad libitum.

Tables XIV to XVI show the results obtained during period II.

The feeding of the pumpkins to both lots was done regularly three times a day as during period I. Pig 630 of lot I, although he was a poor gainer and showed symptoms of indigestion at the end of period I and continued in this condition into period II, was retained to await further development of his illness. During the second week of period II, pig 633 of the control lot (lot II) began to scour and vomit and lost 11 pounds of her weight. The cause of sickness of pig 630, therefore,

could not well be ascribed to the presence of the pumpkins or ground seeds, for pig 633, which received no pumpkin whatsoever and was in better condition, followed the example of pig 630. For some reason unexplained, except possibly by the rainy weather during the last week of period II, none of the lots made progress as expected. At the end of period II the sick pigs, 630 and 633, were discarded. To balance up the lots, pig 640 of lot III was also expelled from the experiment, because of slow development. The remaining 12 animals were then divided into two lots. Lot I, retaining the 4 of the original animals, received pigs 641 and 643 from lot III. Lot II received pigs 626 and 628 from lot III, as an addition to its remaining 4.

TABLE XIV.—*Feed consumed and effect on weight in period II*

Lot No.	Date.	Nutrients per 100 pounds live weight.				Gain or loss per hundredweight.
		Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	Nov. 27 to Dec. 4	6.177	3.105	22.750	1.701	13.05
	Dec. 4 to Dec. 11	5.698	3.291	18.164	1.748	−1.27
	Average per week.	5.937	3.198	20.457	1.724	.....
II	Nov. 27 to Dec. 4	5.170	1.751	23.513	1.016	12.17
	Dec. 4 to Dec. 11	4.549	1.547	20.882	.888	−4.49
	Average per week.	4.859	1.649	22.197	.952	.....
III	Nov. 27 to Dec. 4	5.484	2.160	23.701	1.270	12.75
	Dec. 4 to Dec. 11	4.844	1.968	20.639	1.142	−1.07
	Average per week.	5.164	2.064	22.170	1.206	.....

TABLE XV.—*Feed consumed during period II*

Lot No.	Date.	Corn per hundredweight.	Middlings per hundredweight.	Tankage per hundredweight.	Pumpkin per hundredweight.		"Cannery" seeds per hundredweight.
					Seeds.	Pulp.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	Nov. 27 to Dec. 4	23.71	7.66	2.39	3.58	25.39	2.39
	Dec. 4 to Dec. 11	18.43	6.35	2.11	3.00	20.94	4.23
	Average per week.	21.07	7.00	2.25	3.29	23.16	3.31
II	Nov. 27 to Dec. 4	26.07	7.87	2.46	.....	.....	.....
	Dec. 4 to Dec. 11	23.46	6.58	2.18	.....	.....	.....
	Average per week.	24.76	7.22	2.32	.....	.....	.....
III	Nov. 27 to Dec. 4	25.50	7.62	2.38	1.85	23.88	.....
	Dec. 4 to Dec. 11	22.19	6.34	2.11	3.03	25.51	.....
	Average per week.	23.84	6.98	2.24	2.44	24.69	.....

TABLE XVI.—*Nutrients required per pound of gain*

Lot No.	Date.	Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	Mean weekly temperature.	
						Maximum.	Minimum.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	°F.	°F.
I	Nov. 27 to Dec. 4	0.473	0.237	1.742	0.129	47	35
	Dec. 4 to Dec. 11	.....	.....	.....	.....	44	34
II	Nov. 27 to Dec. 4	.424	.143	1.932	.083	47	35
	Dec. 4 to Dec. 11	.....	.....	.....	.....	44	34
III	Nov. 27 to Dec. 4	.430	.169	1.858	.099	47	35
	Dec. 4 to Dec. 11	.....	.....	.....	.....	44	34

PERIOD III.—This period began December 11 at noon and closed December 24 at noon. Only two lots, I and II, were under experimentation. The object during this period was to find the approximate value of the entire pumpkin as a succulent and the ground "cannery" seeds as a concentrate in the ration for young growing pigs. During this period lot I received chopped pumpkin (containing 10.65 per cent seeds), fed by hand, and a known amount of ground "cannery" seeds mixed with the middlings of the self-feeder ad libitum. Lot II was on the self-feeder only, thus serving as a control.

Tables XVII to XIX show the results obtained during period III.

TABLE XVII.—*Feed consumed and effect on weight in period III*

Lot No.	Date.	Nutrients per 100 pounds live weight.				Gain per hundredweight.
		Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	Dec. 11 to Dec. 18	5.802	2.812	22.311	1.267	10.81
	Dec. 18 to Dec. 24	5.435	2.514	19.343	1.082	8.32
	Average	5.618	2.663	20.827	1.174	9.56
II	Dec. 11 to Dec. 18	5.657	1.839	23.958	1.044	11.34
	Dec. 18 to Dec. 24	4.776	1.446	17.583	.940	2.02
	Average	5.216	1.642	20.720	.992	6.68

During the first week of period III no marked difference in gain and consumption of food per 100 pounds live weight took place. However, during the second week of this period lot I gained four times more weight per hundredweight, consuming a little more of each ingredient.

PERIOD IV.—This period is practically a continuation of the preceding period except for the elimination of pumpkins from the ration. The ground "cannery" seeds were mixed with the middlings and placed in the self-feeder from which the pigs could get it ad libitum. The object

during this period was to find the value of these Cucurbita seeds as a constituent of the dry mash and the possible effects on young pigs when fed ad libitum.

The results of this study are given in Tables XX to XXII.

TABLE XVIII.—*Feed consumed during period III*

Lot No.	Date.	Corn per hundredweight.	Middlings per hundredweight.	Tankage per hundredweight.	Pumpkins per hudredweight.		Cannery seeds per hundredweight.
					Seeds.	Pulp.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I.....	Dec. 11 to Dec. 18..	26. 48	3. 19	2. 93	3. 14	26. 37	1. 92
	Dec. 18 to Dec. 24..	23. 23	2. 26	3. 32	3. 18	26. 70	1. 66
	Average.....	24. 85	2. 72	3. 12	3. 16	26. 53	1. 79
II.....	Dec. 11 to Dec. 18..	26. 71	7. 75	3. 25	.....	.....	.....
	Dec. 18 to Dec. 24..	17. 97	7. 79	3. 14	.....	.....	.....
	Average.....	22. 34	7. 77	3. 19	.....	.....	.....

TABLE XIX.—*Nutrients required per pound of gain*

Lot No.	Date.	Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	Mean weekly temperature.	
						Maximum.	Minimum.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*°F.*	*°F.*
I.....	Dec. 11 to Dec. 18..	0. 536	0. 260	2. 063	0. 117	48	31
	Dec. 18 to Dec. 24..	. 653	. 302	2. 326	. 130	40	26
	Average.....	. 594	. 281	2. 194	. 123	.....	.....
II.....	Dec. 11 to Dec. 18..	. 498	. 162	2. 112	. 092	48	31
	Dec. 18 to Dec. 24..	2. 364	. 715	8. 704	. 465	40	26
	Average.....	1. 431	. 438	5. 408	. 278	.....	.....

TABLE XX.—*Feed consumed and effect on weight in period IV*

Lot No.	Date.	Nutrients per 100 pounds live weight.				Gain per hundredweight.
		Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I.....	Dec. 24 to Dec. 31.....	5. 905	3. 164	18. 074	1. 480	6. 14
II.....	Dec. 24 to Dec. 31.....	5. 260	1. 650	20. 424	1. 002	8. 51

The difference in gain of lot I was attributed to the abrupt change in feeding (excluding the pumpkins which were fed to them continuously

for eight weeks). No ill effects were noticed in lot I. Pig 626 of lot II showed signs of indigestion (loss of appetite). At the close of the experiment the general appearance of the animals of pen No. 1 was more favorable than that of the animals of the control lot (pen No. 1). The original four of lot I made a total gain of 148 pounds throughout the entire experiment against a total gain of 99.75 pounds made by the four original animals of the control lot, leaving a margin of 48.25 pounds in favor of lot I.

The record of each pig is given in Table XXIII.

TABLE XXI.—*Food consumed during period IV*

Lot No.	Date.	Corn per hundredweight.	Middlings per hundredweight.	Tankage per hundredweight.	"Cannery" seeds per hundredweight.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
I	Dec. 24 to Dec. 31	20.53	4.39	3.35	4.95
II	Dec. 24 to Dec. 31	21.73	7.92	3.52	.....

TABLE XXII.—*Nutrients required per pound of gain*

Lot No.	Date.	Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	Mean weekly temperature.	
						Maximum.	Minimum.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*° F.*	*° F.*
I	Dec. 24 to Dec. 31	0.961	0.515	2.943	0.241	38	24
II	Dec. 24 to Dec. 31	.618	.193	2.400	.117	38	24

TABLE XXIII.—*Individual live weights of the pigs*

Lot No.	Tag No.	Sex.	Oct. 30.	Nov. 6.	Nov. 13.	Nov. 20.	Nov. 27.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	626	Female	30	32	36	38.5	42
	627	Barrow	31	32	35	39	45
	628	Female	31	32	37	41	44.5
	629	do	28	31.5	33	36.25	40.5
	630	Barrow	25.5	27.5	29	30.5	30.25
	631	do	35	35.5	37	41.5	48.5
II	632	do	29	30	32	36	38.5
	633	Female	32	34	38	41	45.25
	634	do	39	38	40	41.5	48
	635	Barrow	27	28.5	30	31.5	34.25
	636	Female	32.5	34.5	34	35.5	37.25
	637	Barrow	42.5	47.5	51	61	69.5
III	638	do	42	47	49	56	61.25
	639	Female	26	26.5	25	25	24.5
	640	do	24.5	24.5	25	26	28
	641	Barrow	35	40.5	39	37.5	37.5
	642	Female	34.5	38.5	37	41	43.5
	643	Barrow	27	31.5	33	35.5	41

TABLE XXIII.—*Individual live weights of the pigs*—Continued

Lot No.	Tag No.	Sex.	Dec. 4.	Dec. 11.	Dec. 18.	Dec. 24.	Dec. 31.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	626	Female	48.5	46.75	53	53.5	51.75
	627	Barrow	51	51	59.5	64	71
	628	Female	50	49.75	52	56.5	58
	629	do	51	53	58	63.5	67.5
	630	Barrow	28	21.25	20	.....	.....
	631	do	56	58	62	68	76.5
II	632	do	44.5	48.75	51.5	54.25	62.75
	633	Female	46.5	35.5	31.75	.....	.....
	634	do	55	55	62.5	64.5	71.5
	635	Barrow	41	41.5	45.75	47.5	52.5
	636	Female	41	37	39.5	38.25	40.5
	637	Barrow	.....	.....	.....	.....	.....
III	638	do	67.5	70.75	81.5	82.5	90.5
	639	Female	.....	.....	.....	.....	.....
	640	do	31.5	30	35.25	.....	.....
	641	Barrow	40.5	38.75	44	45.5	45
	642	Female	.....	.....	.....	.....	.....
	643	Barrow	48.5	47.75	55	60.5	62

It is of interest to note that the content of lecithin, a growth-promoting substance, is nearly twice as great in shelled pumpkin seeds as in linseed cake (7).

SUMMARY OF PART II

1. When pumpkins are fed to very young pigs, for some reason the seeds are more readily consumed than the pulp.

2. Whole seeds appeared in feces at first.

3. Although sickness occurred, it could not be attributed to the presence of Cucurbita seeds in the ration.

4. Pumpkins, as a succulent, tend to increase the appetite.

5. Ground "cannery" seeds fed ad libitum have no detrimental effect on the metabolism of young growing pigs.

Because of individuality and other variable factors existing in lot feeding it is not wise to conclude that the feeding of the pumpkin seeds was responsible for any better results obtained, so far as gain, thrift, and general appearance of the animals is concerned. However, it is safe to say that no injurious effects have been noticed on the animals to which the pumpkin seeds were fed, whether in the fruit or when received as a refuse from the canning factory. In the opinion of the writer a period of 62 days with the very young pigs used in this work is sufficient to warrant the conclusion that the existing belief among farmers that pumpkin seeds are injurious to young pigs is groundless.

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A CONSTANT-TEMPERATURE BATH FOR HEATING BLOOD SERUM

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For heating clear anti-hog-cholera serum to a temperature of 58° to 60° C., as recommended by Dorset and Henley,[1] some form of constant-temperature bath is very desirable. Temperatures much higher than 60° are very injurious to serum, and on that account any form of heating bath should have some arrangement to prevent temperatures higher than 60°; indeed, this is of almost fundamental importance in any automatically controlled bath. When the heating bath is kept under constant personal supervision the temperature is easily regulated and kept within the desired range, but with an automatic heater there is supposed to be no necessity for close personal supervision, and a derangement of the temperature-controlling apparatus, if unnoticed, may result in the complete loss of a batch of serum unless some way of preventing temperatures injurious to the serum has been provided.

After considerable experimentation a bath has been devised which seems to embody a number of advantages. The operation of this bath depends upon the utilization of the vapors of a liquid having a boiling point at or about 61° C. as the heating medium, and the bath is so constructed that a very small quantity of the liquid may be used.

OPERATION AND DESCRIPTION OF THE BATH

A diagram of the bath as used in the laboratory is shown in figure 1. In brief, the bath consists of (1) a stand, *A;* (2) a jacketed kettle, *B*, provided with a draw-off valve, *a*, for the inner kettle, an inlet, *b*, and an outlet, *c*, to the outer jacket; (3) a cover, *C;* (4) a stirrer, *D;* (5) a condenser, *E;* (6) a liter flask, *F;* and (7) a thermometer, *T*. A perforated cork stopper, *s*, covered with tin foil, should be used to connect the flask, *F*, to the inlet of the kettle.

In operating, the draw-off valve, *a*, is closed, the inner kettle, *B*, is filled with serum, the cover, *C*, and the stirrer, *D*, are adjusted and started, the condenser, *E*, is attached, and water is allowed to flow through it. About 300 cc. of chloroform (U. S. P., boiling point 61° C.) is placed in the flask, *F*, and the flask is then attached to the inlet, *b*, of the jacket. Heat, preferably a gas flame, or any other source of plentiful, controllable

[1] DORSET, M., and HENLEY, R. R. PRODUCTION OF CLEAR AND STERILIZED ANTI-HOG-CHOLERA SERUM [Preliminary paper.] *In* Jour. Agr. Research, v. 6, no. 9, p. 335-338. 1916.

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heat, is applied to the flask, *F*. Heat should be supplied in abundance in the beginning so as to keep the chloroform boiling constantly and should be reduced only when the condenser capacity is exceeded. As a usual thing there is but little condensation of the chloroform in the condenser until the serum temperature exceeds 52°. In other words, until the temperature of the serum reaches 52° the kettle itself acts as a condenser.

The kettle used in the tests has a capacity of 10 liters. It has been possible to raise the temperature of this quantity of water in one hour from 15° C. to 58°, and a temperature of 58° to 60° has been maintained for six hours with a loss of only 20 cc. of chloroform. With a condenser functioning properly no loss of chloroform should occur.

LIQUIDS SUITABLE FOR HEATING MEDIUM

Of the liquids used, or considered, as heating mediums, chloroform is the most satisfactory, since (1) it has a suitable boiling point, (2) it is noninflammable, (3) it has a characteristic odor, (4) it is not poisonous, (5) it is not corrosive, (6) it is insoluble in water, and (7) it is reasonably cheap and easily obtained. Although the boiling point of chloroform is 61° C., it has never been possible to raise the temperature of the serum in the kettle above 60° even when sufficient heat was applied to overtax the condenser.

In altitudes greater than that of Washington, D. C., it may be necessary to use a liquid of higher sea-level boiling point than chloroform. It is believed that the most suitable liquid for this purpose is a mixture of carbon tetrachlorid and chloroform. Carbon tetrachlorid boils at 74° C. at sea level, and so mixtures of it and chloroform may be prepared with boiling points at sea level varying from a little above 61° to a little below 74°, depending upon the quantity of each liquid present. For example, at an altitude of 5,000 feet the boiling point of chloroform is considerably lower than 61°. In this case a mixture of carbon tetrachlorid and chloroform having a boiling point of 61° should be prepared. The proportion of carbon tetrachlorid to chloroform that will give a mixture having a boiling point of 61° for a given altitude can be easily determined by preparing several mixtures of the two liquids in varying proportions and determining the boiling point of each. In this way the proportion of carbon tetrachlorid to chloroform that is required to give a mixture with boiling point of 61° at the altitude in question may be ascertained.

If a liquid of lower boiling point than 61° C. at sea level should be desired, acetone, which has a boiling point of 58°, is suggested. Acetone, however, is inflammable and is soluble in water.

In all cases where mixtures are used, the utmost precaution should be taken to insure the perfect condensation of the vapors, since otherwise by

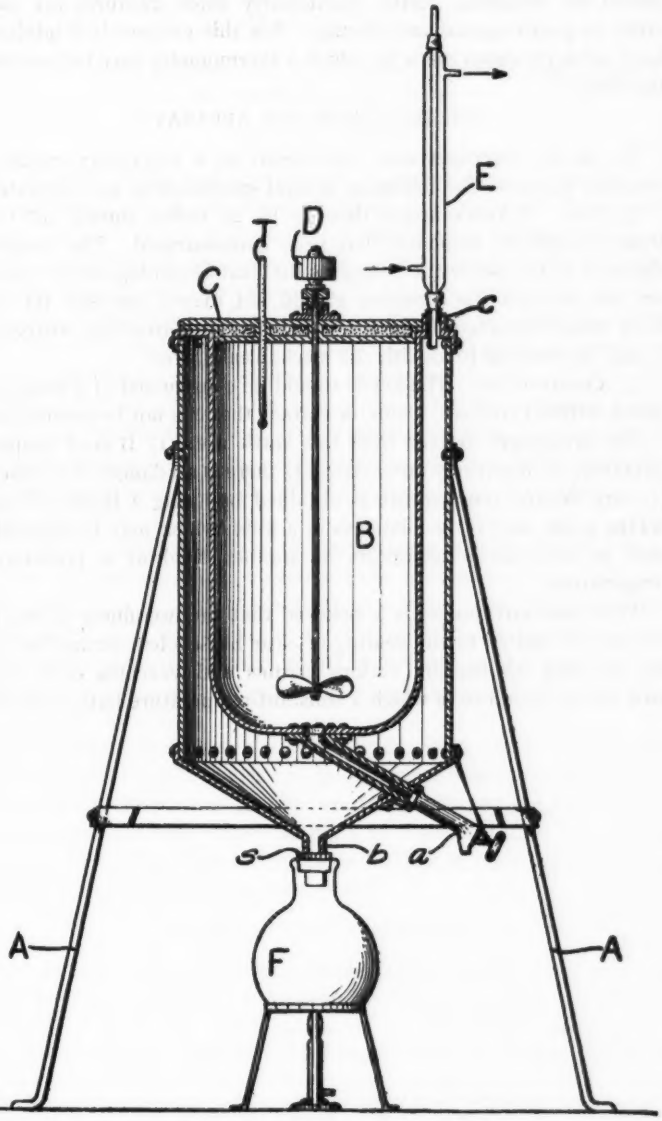


FIG. 1.—Automatic heating bath for anti-hog-cholera serum.

the escape of the more volatile constituent the boiling point of the residue is increased. The boiling point of the liquid used as a heating medium should be frequently tested, particularly when mixtures are used, in order to guard against any change. For this purpose it is advisable to have some provision made by which a thermometer may be inserted into the flask, *F*.

SPECIFICATIONS FOR APPARATUS

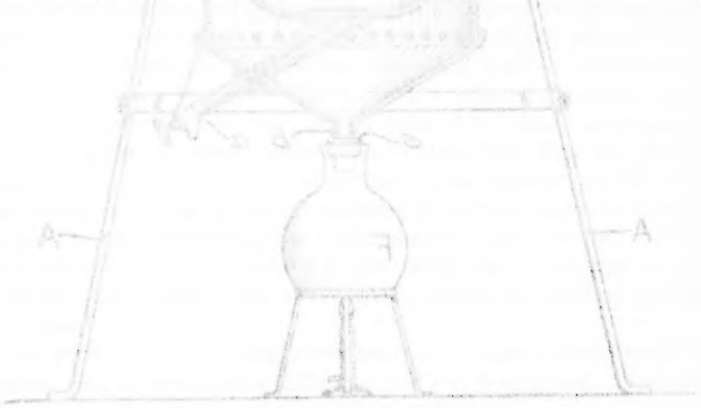
The kettle described was constructed as a laboratory model. For practical purposes the following general specifications are suggested:

(1) Size. A kettle larger than 10 by 20 inches should not be constructed until its feasibility has been demonstrated. The smaller the diameter of the kettle the greater is the heat-absorbing surface obtained. On this account the diameter should not exceed one-half the height. With small diameters there is a rapid flow of convection currents, and it may be possible to abolish the mechanical stirrer.

(2) Construction. The kettle should be constructed of a noncorrosive metal, without crevices, seams, or corners that can not be readily cleaned.

The advantages derived from this kettle are: (1) It is of simple construction, (2) is entirely automatic, (3) there is no danger of overheating, (4) any desired temperature is obtained by using a liquid of suitable boiling point, and (5) a minimum of a fluid, which may be expensive, is used to maintain a maximum of another fluid at a predetermined temperature.

With these advantages it is believed that the usefulness of this kettle will not be limited to the heating of clear hog-cholera serum but that it can be used for heating various serums and vaccines, or it may be used for any purpose in which a constant-temperature bath is necessary.



ASSIMILATION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY CORN WHEN NUTRIENT SALTS ARE CONFINED TO DIFFERENT ROOTS

By P. L. GILE, *formerly Chemist*, and J. O. CARRERO, *Assistant Chemist, Porto Rico Agricultural Experiment Station*

INTRODUCTION

In a former number of the Journal of Agricultural Research,[1] data were presented which showed that when the supply of an essential element is restricted to a portion of a plant's roots the amount of this element assimilated is diminished. The relation between the fraction of the roots supplied and the amount of the element assimilated seemed to agree with Mitscherlich's formulation of the law of minimum. Practically the same factor was obtained for the assimilation of nitrogen as for the assimilation of phosphorus, potassium, or iron. These data, however, were all obtained for one general condition—namely, where part of the plant's roots were in a complete nutrient solution and the remainder were in a nutrient solution lacking only one essential element. No tests were conducted with part of the roots in a solution lacking two or more elements.

The present paper reports work which shows how the assimilations of nitrogen, phosphorus, and potassium were affected when half the roots of the plant were in a complete nutrient solution and half in a solution lacking more than one essential element; when the roots were divided between two solutions, each of which lacked one or two elements; and when the roots were divided among three solutions, each of which lacked one or two elements.

Only nitrogen, phosphorus, and potassium were varied in these tests. It did not seem feasible to attempt to determine how the assimilations of calcium, magnesium, and sodium would be affected by localizing the supply, since relatively small amounts of these elements are absorbed by corn. Moreover, nutrient solutions lacking in these elements would probably be unfavorable for root growth.

METHOD OF EXPERIMENTS

The methods followed in conducting these experiments were very similar to those employed in the tests previously reported.

Corn (*Zea mays* L.), was used in all the following experiments because of the facility with which its roots could be divided among the

[1] GILE, P. L., and CARRERO, J. O. ABSORPTION OF NUTRIENTS AS AFFECTED BY THE NUMBER OF ROOTS SUPPLIED WITH THE NUTRIENT. *In* Jour. Agr. Research, v. 9, no. 3, p. 73-95, 2 fig. 1917. Literature cited, p. 94-95.

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different flasks. The seeds were germinated in sphagnum moss or in thoroughly leached coconut fiber. When the plumules were about 1½ inches long the seedlings were transferred to the nutrient solutions.

Two or three Erlenmeyer flasks joined together at the necks and covered with black cloth were used as containers for the nutrient solutions. One seedling was grown in each of these double or triple flasks for a period of 20 days. The plants were started in 200-cc. flasks, but as they became larger they were transferred successively to 500-cc. and 1,000-cc. flasks. By guiding the new roots into the proper flasks, the roots of each plant were kept equally divided between the two or three nutrient solutions afforded the plant.

The nutrient solutions were renewed six times during the 20-day period of each experiment, and transpired water was replaced daily. While the plants were small the solutions were changed every 4 days; later the solutions were changed every 3 days, and finally every 2 days. The frequent renewals of the solutions and the large size of the flasks insured an ample supply of the nutrients.

Rain water, caught on the roof of the glasshouse, was used in making up the nutrient solutions. It contained only 17 parts per million of total solids (organic and inorganic) and was, therefore, sufficiently pure for these experiments. In these experiments it was necessary only to guard against appreciable contamination with nitrogen, phosphoric acid, and potash. The compositions of the nutrient solutions used in the different tests are given in Table I.

TABLE I.—*Composition of nutrient solutions used*

Chemical.	Complete solution.	Solution lacking nitrogen.	Solution lacking phosphorus.	Solution lacking potassium.	Solution lacking nitrogen and phosphorus.	Solution lacking nitrogen and potassium.	Solution lacking phosphorus and potassium.	Solution lacking nitrogen, phosphorus, and potassium.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Monopotassium phosphate (KH_2PO_4)	7.14	7.14	.....	.....	.....	.....	.....	.....
Monosodium phosphate (NaH_2PO_4)	.....	.....	.....	10.06	.....	10.05	.....	.....
Potassium nitrate (KNO_3)	14.40	.....	14.40	.....	.....	.....	.....	.....
Sodium nitrate ($NaNO_3$)	9.18	.....	9.18	15.20	.....	.....	.....	.....
Calcium nitrate ($Ca(NO_3)_2 4H_2O$)	12.78	.....	12.78	21.14	.....	.....	.....	.....
Potassium sulphate (K_2SO_4)	.....	12.40	4.56	.....	16.96	.....	.....	.....
Sodium sulphate ($Na_2SO_4 10H_2O$)	3.16	19.86	2.44	.....	19.83	11.60	19.85	19.85
Calcium chlorid ($CaCl_2 6H_2O$)	.....	11.84	.....	.....	11.84	11.84	11.84	11.84
Magnesium chlorid ($MgCl_2 6H_2O$)	4.50	.....	4.50	4.50	.....	.....	.....	.....
Magnesium sulphate ($MgSO_4 7H_2O$)	.....	4.06	.....	2.66	4.06	4.06	4.06	4.06
Ferric tartrate ($Fe_2(C_4H_4O_6)_3 + H_2O$)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Calcium carbonate, precipitated, ($CaCO_3$)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Water	100,000 cc.							

Precipitated calcium carbonate was used in all solutions, since preliminary tests showed that it measurably increased the growth of roots in certain incomplete solutions. Ferric tartrate was used as the source of iron, previous work having shown this to be an especially available form of iron in the presence of calcium carbonate. In all the nutrient solutions, except that lacking potassium, the bases were in the following proportions by weight: 1 Mg to 4 Ca to 5.3 Na to 14 K. In the solution lacking potassium the proportions were 1 Mg to 4 Ca to 6 Na.[1]

The nutrient solutions, with the exception of iron, were made up 18 hours before they were to be used. Ferric tartrate was added just before the solutions were used, since the availability of the iron in the solution decreases somewhat with time.

All the plants grown in the experiments were analyzed, by the usual analytical methods, for nitrogen, phosphoric acid, and potash.[2] The results reported are, in practically every case, the average of closely agreeing duplicate or triplicate determinations.

In each experiment one lot of plants was grown with all the roots in the complete nutrient solution, and another lot was grown with all the roots in the solution lacking nitrogen, phosphorus, and potassium. The normal, or maximum, assimilation of nitrogen, phosphorus, or potassium for any experiment was taken as the difference between the quantities present in these two lots of plants. The assimilations attained by plants having their roots divided between more or less complete solutions [3] were expressed relative to this maximum or normal assimilation.

In all the experiments 1 corn plant was grown in each double or triple flask, and 8 plants were taken as a unit, the units being duplicated for each separate treatment. There were, therefore, 16 plants which were treated alike in every case.

EXPERIMENTAL RESULTS

PLANTS GROWN WITH THEIR ROOTS EQUALLY DIVIDED BETWEEN TWO SOLUTIONS

EXPERIMENT 1.—The roots divided between a complete solution and a solution lacking two elements.

Since in this work only nitrogen, phosphorus, and potassium are varied, there are evidently only three possible pairs of solutions which

[1] The small amount of calcium resulting from the slight solubility of calcium carbonate was not considered in calculating these proportions.

[2] Nitrogen and potash were determined in the roots as well as in the stalks and leaves, since the roots could be washed free from nitrates and potash salts. Phosphoric acid, however, was determined only in the stalks and leaves, because it seemed probable that some precipitate of calcium or ferric phosphate might adhere to the roots, even after thorough washing.

[3] The amount of nitrogen, phosphoric acid, or potash assimilated by these plants was taken as the amount present in the plants minus the amount present in the plants grown in the solution lacking nitrogen, phosphorus, and potassium.

consist of a complete solution and one lacking in two elements. Data on the growth of plants in these three pairs of solutions are given in Table II, and data on the assimilation of nitrogen, phosphoric acid, and potash in Table III.

The plants having half their roots in incomplete solutions had slightly higher ratios of roots to tops than the control plants, which had all their roots in the complete solution.[1] It is also noticeable that plants 33 to 80 showed a greater growth of roots in the A flasks, which contained the complete solution, than in the B flasks containing the incomplete solutions.

The means of the amounts of nitrogen, phosphoric acid, and potash assimilated by plants 33 to 48, 49 to 64, and 65 to 80 were almost identical. The relative amounts of nitrogen, phosphoric acid, and potash assimilated by these three sets of plants varied, however, according to the character of the incomplete solution in flask B.

EXPERIMENT II.—The roots divided between a complete solution and a solution lacking three elements.

The data on growth and on the assimilation of nitrogen, phosphoric acid, and potash, along with the results of experiment VIII, are given in Tables XIV and XV.

While plants 49 to 64 had almost the same numbers of roots in the A and B flasks, nearly two-thirds of the total root growth was in the A flask containing the complete solution. The roots in the complete solution were much shorter and more bushy than those in the solution lacking nitrogen, phosphorus, and potassium.

The proportions in which nitrogen, phosphorus, and potassium were assimilated by plants 49 to 64 differed but little from the proportions assimilated by the control plants, 1 to 16, although the mean assimilation of these three elements by plants 49 to 64 was only about two-thirds that of the controls. It would be interesting to know whether assimilation would be cut to 50 per cent if one-half the roots were maintained in water containing no nutrients whatever—that is, no sodium, calcium, magnesium, iron, chlorids, and sulphates. Because of the difficulty of preparing sufficient quantities of suitable distilled water, this was not determined.

EXPERIMENT III.—The roots divided between two solutions, each of which lacked one element.

The three nutrient solutions lacking in nitrogen, phosphorus, and potassium, respectively, may be combined in three pairs. The growth made in these three pairs of solutions, and the analyses of the plants are shown in Tables IV and V. It will be noted that the growths made by the plants in each of the three pairs of incomplete solutions varied markedly. Roughly, the root to top ratios were inversely proportional to the growths of tops.

[1] The root to top ratio is taken as the weight of dry roots divided by the weight of dry leaves and stalks (tops).

TABLE II.—*Growth of plants in experiment I*

Plant No.	Nutrient solution.		Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Average oven-dry weight per root.		Ratio of roots to tops.	Percentage of total roots by weight.	
	A flasks.	B flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	A flasks.	B flasks.	A flasks.	B flasks.		A flasks.	B flasks.
			Gm.	*Gm.*	*Gm.*			*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1 to 8	Complete (NPK present)	Complete (NPK present)	241. 78	19. 01	19. 31	88	98	2. 39	2. 40	0. 0191	0. 0248	0. 252	50. 5	49. 5
9 to 16			247. 8	19. 6		97	98	2. 38	2. 28			. 238		
17 to 24	NPK lacking	NPK lacking	15. 85	2. 26	2. 18	58	63	. 83	. 79	. 0126	. 0133	. 717	50. 0	50. 0
25 to 32			15. 54	2. 09		64	66	. 83	. 88			. 818		
33 to 40	Complete (NPK present)	PK lacking (N present)	206. 42	16. 79	18. 00	89	92	2. 43	2. 26	. 0284	. 0262	. 279	52. 0	48. 0
41 to 48			229. 43	19. 21		90	87	2. 69	2. 45			. 268		
49 to 56	Complete (NPK present)	NK lacking (P present)	218. 56	18. 11	17. 85	96	100	2. 77	1. 88	. 0289	. 0193	. 257	61. 0	39. 0
57 to 64			224. 42	17. 59		99	87	2. 88	1. 73			. 262		
65 to 72	Complete (NPK present)	NP lacking (K present)	227. 57	18. 64	18. 51	93	94	2. 77	1. 97	. 0302	. 0205	. 254	58. 3	41. 7
73 to 80			225. 8	18. 38		93	102	2. 84	2. 05			. 266		

TABLE III.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment I*

Plant No.	Nutrient solution.		Nutrients present in oven-dry stalks and leaves.			Nutrients present in oven-dry roots.				Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
						Nitrogen (N).		Potash (K_2O).					
	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*				
1 to 16	Complete (NPK present)	Complete (NPK present)	3.81	1.53	8.18	2.47	2.47	5.37	5.37	100	100	100	100
17 to 32	NPK lacking	NPK lacking	1.21	.29	1.45	1.12	1.12	.98	.98				
33 to 48	Complete (NPK present)	PK lacking (N present)	4.19	1.3	6.6	2.79	2.7	5.07	2.11	105	79	74	86
49 to 64	Complete (NPK present)	NK lacking (P present)	3.73	1.51	7.51	2.69	1.57	4.66	2.53	90	91	82	88
65 to 80	Complete (NPK present)	NP lacking (K present)	3.56	1.34	7.72	2.61	1.33	4.55	4.95	88	84	90	87

TABLE IV.—*Growth of plants in experiment III*

Plant No.	Nutrient solution.		Weight of stalks and leaves.			Number of roots.		Oven-dry weight of roots.		Average oven-dry weight per root.		Ratio of roots to tops.	Percentage of total roots by weight.	
	A flasks.	B flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	A flasks.	B flasks.	A flasks.	B flasks.		A flasks.	B flasks.
			Gm.	*Gm.*	*Gm.*			*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1 to 8	Complete (NPK present)	Complete (NPK present)	265. 99	21. 66	23. 56	100	100	2. 43	2. 50	0. 0256	0. 0255	0. 227	49. 2	50. 8
9 to 16			307. 7	25. 45		94	101	2. 52	2. 61			. 202		
17 to 24	NPK lacking	NPK lacking	11. 66	3. 36	3. 3	63	67	. 98	. 96	. 0153	. 0149	. 577	50. 2	49. 8
25 to 32			11. 35	3. 24		69	70	1. 04	1. 04			. 642		
33 to 40	N lacking (PK present)	P lacking (NK present)	125. 51	12. 21	12. 05	100	93	2. 22	2. 93	. 0220	. 0305	. 422	42. 5	57. 5
41 to 48			120. 17	11. 86		92	94	2. 00	2. 77			. 402		
49 to 56	N lacking (PK present)	K lacking (NP present)	166. 15	14. 87	16. 19	94	95	1. 85	2. 82	. 0196	. 0288	. 314	38. 9	61. 2
57 to 64			200. 22	17. 51		97	109	1. 88	3. 05			. 282		
65 to 72	K lacking (NP present)	P lacking (NK present)	220. 14	19. 89	20. 87	99	100	2. 62	2. 58	. 0278	. 0259	. 261	52. 6	47. 4
73 to 80			253. 13	21. 85		107	99	3. 09	2. 58			. 259		

TABLE V.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment III*

Plant No.	Nutrient solution.		Nutrients present in oven-dry leaves and stalks.			Nutrients present in oven-dry roots.				Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
						Nitrogen (N).		Potash (K_2O).					
	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*				
1 to 16	Complete (NPK present)	Complete (NPK present)	3.73	1.62	7.69	2.58	2.58	5.66	5.66	100.0	100.0	100.0	100.0
17 to 32	NPK lacking	NPK lacking	1.17	.32	2.13	1.24	1.24	1.87	1.87				
33 to 48	N lacking (PK present)	P lacking (NK present)	2.92	.62	6.44	1.43	2.61	4.58	5.41	42.6	17.3	46.3	35.4
49 to 64	N lacking (PK present)	K lacking (NP present)	3.56	1.63	5.08	1.86	2.97	4.84	1.89	67.2	68.3	43.3	59.6
65 to 80	K lacking (NP present)	P lacking (NK present)	3.91	1.4	5.78	3.01	2.8	1.88	5.26	96.4	75.9	64.8	79.0

In the two preceding experiments and in the work reported in the previous paper, where half the roots were in a complete solution, the lack of any one element did not significantly depress growth and assimilation more than the lack of another. In this experiment, however, where both the solutions were incomplete, growth and assimilation varied markedly according to which elements were supplied to only half the roots. A restriction of the supply of nitrogen and phosphorus to separate halves of the roots depressed growth and assimilation more than a similar restriction of the supply of nitrogen and potassium, or phosphorus and potassium.

The relative growths of roots in the A and B flasks, shown by the relative percentages of total roots by weight, in the two flasks is of interest. If the relative growths are indicative of the relative needs of the plant for the elements present in the solution, it would seem from the data on plants 33 to 48 that nitrogen is needed more than phosphorus; from plants 49 to 64, that nitrogen is needed much more than potassium; and from plants 65 to 80, that phosphorus is needed slightly more than potassium. The relative needs of the plants for these three elements may well hold for corn only and for this stage of growth of corn.

EXPERIMENT IV.—The roots divided between two solutions, one of which lacked one element and the other two elements.

The three nutrient solutions lacking nitrogen, phosphorus, and potassium, respectively, and the three solutions lacking nitrogen and phosphorus, nitrogen and potassium, and phosphorus and potassium, respectively, can be combined in nine pairs. Only three of the possible combinations, however, afford in the pair a complete nutrient solution. The effect of these three pairs of solutions on growth and the assimilation of nutrients are shown in Tables VI and VII.

The assumption made in the previous experiment, that corn needs nitrogen more than it needs phosphorus, and phosphorus slightly more than potassium during the period of growth covered by these tests, helps to explain the results obtained in this experiment as well as the results obtained in the succeeding ones. A second assumption, however, is needed—namely, the assimilation of nitrogen, phosphorus, or potassium is greater when these elements are absorbed from solutions containing two or all three of them than when they are absorbed from solutions containing only one of them.

This second assumption explains why plants 49 to 64 assimilated relatively more nitrogen than plants 33 to 48 and relatively more phosphorus than plants 65 to 80, also why plants 65 to 80 assimilated relatively more potassium than plants 49 to 64.

TABLE VI.—*Growth of plants in experiment IV*

Plant No.	Nutrient solution.		Weight of stalks and leaves.			Number of roots.		Oven-dry weight of roots.		Average oven-dry weight per root.		Ratio of roots to tops.	Percentage of total roots by weight.	
	A flasks.	B flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	A flasks.	B flasks.	A flasks.	B flasks.		A flasks.	B flasks.
			Gm.	*Gm.*	*Gm.*			*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1 to 8	Complete (NPK present)	Complete (NPK present)	227.22	18.21	19.65	91	80	2.46	2.13	0.0274	0.0249	0.251	53.4	46.6
9 to 16			242.37	21.09		89	94	2.47	2.21			.222		
17 to 24	NPK lacking	NPK lacking	18.57	2.44	2.54	55	50	.81	.79	.0148	.0158	.656	51.2	48.8
25 to 32			19.57	2.63		56	50	.85	.78			.62		
33 to 40	N lacking (PK present)	PK lacking (N present)	97.05	9.21	10.33	74	80	1.52	2.27	.0228	.0301	.412	42.5	57.5
41 to 48			133.32	11.45		82	80	2.04	2.55			.401		
49 to 56	K lacking (NP present)	NP lacking (K present)	174.36	14.75	14.01	90	89	3.00	1.85	.0303	.0199	.329	61.4	38.6
57 to 64			150.56	13.26		85	84	2.51	1.60			.31		
65 to 72	P lacking (NK present)	(NK lacking (P present)	116.65	10.9	10.92	81	85	2.70	1.94	.0305	.0223	.426	56.3	43.7
73 to 80			115.60	10.94		80	87	2.24	1.90			.379		

TABLE VII.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment IV*

Plant No.	Nutrient solution.		Nutrients present in oven-dry stalks and leaves.			Nutrients present in oven-dry roots.				Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
						Nitrogen (N).		Potash (K_2O).					
	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*				
1 to 16	Complete (NPK present)	Complete (NPK present)	4.07	1.46	7.45	2.68	2.68	4.91	4.91	100.0	100.0	100.0	100.0
17 to 32	PK lacking	NPK lacking	1.13	.49	2.6	1.24	1.24	2.13	2.13	.....	.....	.....	.....
33 to 48	N lacking (PK present)	PK lacking (N present)	3.60	.83	5.03	1.72	2.92	4.77	1.78	48.5	28.0	35.8	37.4
49 to 64	K lacking (NP present)	NP lacking (K present)	3.64	1.31	4.44	3.00	1.64	1.78	4.24	65.4	62.7	41.8	56.6
65 to 80	P lacking (NK present)	NK lacking (P present)	3.00	.61	6.30	2.58	1.58	4.85	2.79	42.6	20.4	48.9	37.3

PLANTS GROWN WITH THEIR ROOTS EQUALLY DIVIDED AMONG THREE SOLUTIONS

It was of interest to see whether there would be a greater depression in growth and assimilation when the roots were divided among three solutions than when the roots were divided between two solutions.

EXPERIMENT V.—The roots divided among three solutions, each of which lacked one element.

In this experiment the roots were equally divided among three solutions which were lacking in nitrogen, phosphorus, and potassium, respectively. The data on growth and assimilation are given in Tables VIII and IX.

A division of the roots among three solutions, each of which lacked one element, depressed the growth by 13 per cent, and the mean assimilation of nitrogen, phosphorus, and potassium, by 32 per cent. In experiment III, where the roots were divided between two solutions, each also lacking in one element, the average depression in growth was 30 per cent and the average depression in the mean assimilation of nitrogen, phosphorus, and potassium was 42 per cent. The plants in experiment V may have had somewhat of an advantage over those in experiment III, since any one of the three elements was available to two-thirds of the roots in this experiment, while in experiment III two of the three elements were each available to only one-half of the roots.

The greatest growth of roots was made in the solution containing nitrogen and phosphorus (C flask), and the least growth took place in the solution containing phosphorus and potassium. These growths agree with the results shown in experiments III and IV.

It is interesting to note that the roots which were in the solution containing nitrogen and phosphorus were considerably richer in nitrogen than those which were in the solution containing nitrogen and potassium; also, the roots which were in the solution containing nitrogen and potassium were slightly richer in potassium than the roots which were in the solution containing phosphorus and potassium. These facts appear to confirm the results of the former experiments in showing that the need of the plants for nitrogen, phosphorus, and potassium is in the order given; and they also seem to indicate that the assimilation of one element from a solution facilitates the assimilation of another element from that solution. The roots in C flask, for example, were richer in nitrogen than those in B flask, because phosphorus is needed more than potassium, and on this account the assimilation of phosphorus aided the assimilation of nitrogen more than did the assimilation of potassium.[1]

EXPERIMENT VI.—The roots divided among three solutions, one of which lacked two elements and two of which each lacked one element.

[1] In this explanation it is assumed that a higher percentage of an element in the root at the time of analysis means a more active assimilation of this element by the root.

TABLE VIII.—*Growth of plants in experiment V*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8.	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	298.5	22.82	23.44	63	62	63	1.90	1.71	1.89	0.0318	0.0293	0.0297	35.6	31.1	33.3	0.241
9 to 16.				320.5	24.05		60	55	60	2.00	1.70	1.76							.227
17 to 24.	NPK lacking...	NPK lacking...	NPK lacking...	8.2	1.28	1.21	32	37	38	.44	.46	.46	.0147	.0129	.0127	33.8	33.8	32.4	1.063
25 to 32.				7.4	1.13		28	31	27	.44	.42	.38							1.097
33 to 40.	N lacking (PK present).	P lacking (NK present).	K lacking (NP present).	247.9	21.93	20.28	66	67	64	2.07	2.31	2.53	.0298	.0352	.0384	29.3	34.3	36.6	.315
41 to 48.				215.5	18.62		61	59	59	1.72	2.12	2.20							.324

TABLE IX.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment V*

Plant No.	Nutrient solution.			Nutrients present in oven-dry stalks and leaves.			Nutrients present in oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16.	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	4.37	1.77	8.72	2.67	2.67	2.67	6.01	6.01	6.01	100	100	100	100
17 to 32.	NPK lacking.....	NPK lacking.....	NPK lacking.....	1.28	.77	.63	.....	.....	.....	.39	.39	.39	.....	.....	.....	.....
33 to 48.	N lacking (PK present).	P lacking (NK present).	K lacking (NP present).	3.89	1.21	6.18	1.65	2.45	3.16	5.21	5.61	2.43	81	58	64	68

When only nitrogen, phosphorus, and potassium are varied, there are nine possible combinations of two solutions lacking one element with one solution lacking two elements. The effects of two of these combinations on the growth of corn and the assimilation of nutrients are shown in Tables X and XI.

In the relative growths made by plants 33 to 48 and 49 to 64 there is further evidence that nitrogen is the element most needed by corn at this stage of growth. Plants 49 to 64 made the greater growth, doubtless because two-thirds of their roots were supplied with nitrogen, while only one-third of the roots of plants 33 to 48 were so supplied.

The relative growths of roots in the A, B, and C flasks present no unusual features; they are evidently dependent on the relative needs of the plant for nitrogen, phosphorus, and potassium, and on the proportions of the roots which were supplied with these three elements.

EXPERIMENT VII.—The roots divided among three solutions, one of which lacked one element and two of which lacked two elements.

Of the solutions named above, six combinations afford a complete nutrient solution; only two of the combinations, however, were tested. The results are shown in Tables XII and XIII.

Plants 49 to 64 contained more nitrogen and phosphorus than plants 33 to 48, since they had an advantage in assimilating these elements from the same solution.

EXPERIMENT VIII.—The roots divided among three solutions, each of which lacked two elements.

In this experiment the nitrogen was in one flask, the phosphorus in another, and the potassium in a third. The results are given in Tables XIV and XV.

The depressions in growth and assimilation and the increase in the root to top ratio were greater in this experiment than in any of the preceding ones.

The distribution of nitrogen and potassium between the roots and tops in this experiment may be of some significance; it seems to support the explanation offered later of the manner in which assimilation is depressed by a division of the roots between incomplete solutions. In the case of plants 33 to 48, the roots which were in the solution containing nitrogen have a higher percentage of nitrogen than the stalks and leaves; also, the roots from the potassium solution are richer in potassium than the tops. The control plants (1 to 16), however, have far higher percentages of nitrogen and potassium in the stalks and leaves than in the roots. In all the preceding experiments (except plants 49 to 64, experiment VII), where assimilation was depressed to a less extent than in this experiment, the stalks and leaves are richer in nitrogen and potassium than the roots.

TABLE X.—*Growth of plants in experiment VI*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	274.6	24.72	24.15	79	68	67	2.33	2.10	2.13	0.0316	0.0300	0.0301	36.3	32.1	32.7	0.265
9 to 16				262.7	23.57		71	75	79	2.40	2.21	2.26							.291
17 to 24	NPK lacking...	NPK lacking...	NPK lacking...	23.0	3.77	3.86	45	48	46	.69	.75	.75	.0136	.0144	.0147	31.2	34.4	34.4	.581
25 to 32				23.3	3.94		55	55	56	.67	.75	.74							.548
33 to 40	K lacking (NP present).	N lacking (PK present).	NP lacking (K present).	110.6	11.96	11.55	64	60	65	2.35	1.32	1.30	.035	.0218	.0212	44.2	27.9	27.9	.416
41 to 48				100.9	11.13		60	66	64	1.99	1.42	1.43							.435
49 to 56	K lacking (NP present).	P lacking (NK present).	NK lacking (P present).	175.5	18.39	18.36	72	71	68	2.35	2.64	1.57	.0362	.0368	.0232	36.9	39.0	24.2	.357
57 to 64				182.0	18.33		65	71	72	2.59	2.58	1.67							.373

TABLE XI.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment VI*

Plant No.	Nutrient solution.			Nutrients present in the oven-dry stalks and leaves.			Nutrients present in oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	3.79	1.55	7.75	2.53	2.53	2.53	5.40	5.40	5.40	100	100	100	100
17 to 32	NPK lacking.....	NPK lacking.....	NPK lacking.....	1.07	.33	1.08	1.32	1.32	1.32	.82	.82	.82	.....	.....	.....	.....
33 to 48	K lacking (NP present).	N lacking (PK present).	NP lacking (K present).	2.85	1.06	4.41	2.68	1.48	1.45	1.85	2.69	4.33	35	30	27	31
49 to 64	K lacking (NP present).	P lacking (NK present).	NK lacking (P present).	3.31	.88	4.97	2.66	2.47	1.60	1.80	4.56	1.70	68	41	48	52

TABLE XII.—*Growth of plants in experiment VII*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8...	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	328.8	26.28	26.13	69	65	70	1.77	1.59	1.88	0.0257	0.0248	0.0257	33.0	32.8	34.2	0.199
9 to 16..				315.4	25.98		70	77	68	1.62	1.78	1.66							.195
17 to 24.	NPK lacking...	NPK lacking...	NPK lacking...	23.6	3.44	3.43	59	47	59	.78	.54	.84	.0136	.012	.0132	34.4	28.2	37.4	.628
25 to 32.				25.3	3.41		47	51	61	.66	.63	.73							.592
33 to 40.	N lacking (PK present).	PK lacking (N present).	NK lacking (P present).	152.8	14.29	13.74	70	66	73	1.64	2.16	1.62	.0238	.0311	.021	32.0	39.8	28.2	.379
41 to 48.				137.8	13.19		66	64	62	1.60	1.88	1.23							.357
49 to 56.	K lacking (NP present).	NP lacking (K present).	NK lacking (P present).	149.9	14.26	14.08	65	58	65	2.25	1.25	1.27	.0345	.0208	.0188	46.6	26.8	26.6	.334
57 to 64.				147.7	13.90		64	66	70	2.22	1.33	1.29							.348

TABLE XIII.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment VII*

Plant No.	Nutrient solution.			Nutrients present in the oven-dry stalks and leaves.			Nutrients present in the oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16...	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	3.92	1.96	7.83	2.55	2.55	2.55	5.71	5.71	5.71	100	100	100	100
17 to 32..	NPK lacking.....	NPK lacking.....	NPK lacking.....	1.37	.29	1.24	1.37	1.37	1.37	.86	.86	.86	.....	.....	.....	.....
33 to 48..	N lacking (PK present).	PK lacking (N present).	NK lacking (P present).	3.25	.92	4.49	1.47	2.82	1.45	4.24	1.66	1.54	45	23	30	33
49 to 64..	K lacking (NP present).	NP lacking (K present).	NK lacking (P present).	3.45	1.43	3.75	3.04	1.62	1.62	1.49	4.44	1.39	49	38	26	38

TABLE XIV.—*Growth of plants in experiments II and VIII*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	437.4	35.85	33.22	75	70	74	3.01	2.65	2.88	0.0359	0.0354	0.0342	34.6	33.1	32.3	0.238
9 to 16				389.6	30.59		76	77	71	2.41	2.53	2.18							.233
17 to 24	NPK lacking	None	None	8.9	2.15	2.04	152	.....	.....	1.67	.....	.....	.0109	.....	.....	.....	.....	.....	.777
25 to 32				6.8	1.92		130	.....	.....	1.40	.....	.....							.729
33 to 40	PK lacking (N present).	NK lacking (P present).	NP lacking (K present).	98.1	11.60	11.38	73	65	64	2.40	1.82	1.95	.0373	.0258	.0272	42.3	27.7	30.1	.532
41 to 48				92.9	11.15		75	66	72	2.74	1.55	1.72							.539
49 to 56	Complete (NPK present).	NPK lacking	None	274.1	22.15	23.65	87	92	.....	4.28	2.49	.....	.0455	.0267	.....	62.7	37.4	.....	.296
57 to 64				287.2	24.44		106	103	.....	4.42	2.70	.....							.291

TABLE XV.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiments II and VIII*

Plant No.	Nutrient solution.			Nutrients present in the oven-dry stalks and leaves.			Nutrients present in the oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	3.98	1.59	8.08	2.46	2.46	2.46	5.53	5.53	5.53	100	100	100	100
17 to 34	NPK lacking.....	None.....	None.....	1.23	.52	.97	1.15	1.15	1.15	.66	.66	.66	.....	.....	.....	.....
35 to 48	PK lacking (N present).	NK lacking (P present).	NP lacking (K present).	2.42	.43	2.86	2.68	1.50	1.65	1.65	1.42	4.15	24	7	14	15
49 to 64	Complete (NPK present).	NPK lacking.....	None.....	3.81	1.41	7.46	2.61	1.59	.....	5.31	2.67	.....	69	62	66	66

TABLE XVI.—*Growth of plants in experiment IX*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	318. 7	26. 51	26. 72	71	75	63	1. 87	2. 15	2. 09	0. 0267	0. 0279	0. 0298	31. 5	35. 3	33. 3	. 023
9 to 16				320. 9	26. 92		71	78	73	1. 92	2. 10	1. 92							. 221
17 to 24	NPK lacking...	NPK lacking..	NPK lacking..	14. 5	2. 41	2. 49	52	45	54	. 60	. 50	. 65	. 0122	. 013	. 0128	32. 4	33. 5	34. 1	. 726
25 to 32				15. 7	2. 57		43	45	40	. 53	. 67	. 54							. 677
33 to 40	Complete (NPK present).	N lacking (PK present).	K lacking (NP present).	250. 6	22. 42	22. 91	70	62	66	2. 29	1. 43	2. 05	. 0293	. 0215	. 029	38. 3	25. 5	36. 1	. 257
41 to 48				270. 0	23. 40		71	66	69	1. 83	1. 31	1. 85							. 209
49 to 56	P lacking (NK present).	N lacking (PK present).	PK lacking (N present).	168. 8	17. 43	17. 28	74	70	72	2. 42	1. 88	2. 37	. 0333	. 0276	. 0309	36. 6	29. 3	34. 1	. 382
57 to 64				167. 9	17. 13		71	70	74	2. 4	1. 98	2. 13							. 38
65 to 72	P lacking (NK present).	NK lacking (P present).	PK lacking (N present).	107. 7	12. 48	12. 61	70	62	64	1. 78	1. 43	1. 59	. 0286	. 0231	. 0248	37. 9	28. 4	33. 7	. 385
73 to 80				107. 8	12. 73		65	59	70	1. 96	1. 36	1. 73							. 397

TABLE XVII.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment IX*

Plant No.	Nutrient solution.			Nutrients present in the oven-dry stalks and leaves.			Nutrients present in the oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	3.41	1.54	7.23	2.36	2.36	2.36	4.51	4.51	4.51	100	100	100	100
17 to 32	NPK lacking....	NPK lacking.....	NPK lacking.....	1.02	.55	1.09	1.07	1.07	1.07	.70	.70	.70	.....	.....	.....	.....
33 to 48	Complete (NPK present).	N lacking (PK present).	K lacking (NP present).	3.33	1.38	5.83	2.37	1.55	2.32	3.99	4.43	1.77	83	76	68	76
49 to 64	P lacking (PK present).	N lacking (PK present).	PK lacking (N present).	2.58	.47	4.86	2.05	1.36	2.10	4.28	3.82	1.85	52	17	46	38
65 to 80	P lacking (NK present).	NK lacking, P present).	PK lacking (N present).	2.21	.39	3.87	2.08	1.36	2.01	3.89	1.76	1.86	32	9	27	23

EXPERIMENT IX.—The roots divided among three solutions. Various combinations of solutions compared.

It is quite evident from a comparison of the results of the preceding experiments that the depressions in growth and absorption were roughly proportional to the incompleteness of the solutions afforded the roots. This is further demonstrated in the present experiment where solutions lacking in different numbers of nutrients are directly compared. The results are given in Tables XVI and XVII.

SUMMARY OF EXPERIMENTAL RESULTS

The important experimental data of all the tests are gathered together in Table XVIII. Since only nitrogen, phosphorus, and potassium were varied in these tests (calcium, magnesium, sodium, iron, sulphate, and chlorid being present in all the solutions), the different nutrient solutions are designated in Table XVIII by their content of nitrogen, phosphorus, and potassium. NP, for example, refers to the solution lacking potassium, and NPK stands for the complete solution, while O represents the solution lacking the three elements. The different combinations of solutions tested are arranged in Table XVIII with the idea of facilitating comparisons.

TABLE XVIII.—*Summary of results of experiments I to IX*

Experiment No.	Solution.			Weight per root.			Percentage of total roots by weight.		
	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.
				Gm.	*Gm.*	*Gm.*			
I	NPK	N	.....	0.0284	0.0262	.....	52.0	48.0	.....
I	NPK	P	.....	.0289	.0193	.....	61.0	39.0	.....
I	NPK	K	.....	.0302	.0205	.....	58.3	41.7	.....
II	NPK	O	.....	.0455	.0267	.....	62.7	37.4	.....
III	PK	NK	.....	.022	.0305	.....	42.5	57.5	.....
III	PK	NP	.....	.0196	.0288	.....	38.9	61.2	.....
III	NP	NK	.....	.0278	.0259	.....	52.6	47.4	.....
IV	PK	N	.....	.0228	.0301	.....	42.5	57.5	.....
IV	NP	K	.....	.0303	.0199	.....	61.4	38.6	.....
IV	NK	P	.....	.0305	.0223	.....	56.3	43.7	.....
IX	NPK	PK	NP	.0293	.0215	0.029	38.3	25.5	36.1
V	PK	NK	NP	.0298	.0352	.0384	29.3	34.3	36.6
IX	NK	PK	N	.0333	.0376	.0309	36.6	29.3	34.1
VI	NP	PK	K	.035	.0218	.0212	44.2	27.9	27.9
VI	NP	NK	P	.0362	.0368	.0232	36.9	39.0	24.2
IX	NK	P	N	.0286	.0231	.0248	37.9	28.4	33.7
VII	PK	N	P	.0238	.0311	.021	32.0	39.8	28.2
VII	NP	K	P	.0234	.0308	.0188	46.6	26.8	26.6
VIII	N	P	K	.0373	.0258	.0272	42.3	27.7	30.1

TABLE XVIII.—*Summary of results of experiments I to IX*—Continued

Experiment No.	Ratio of roots to tops relative to that of controls taken as 100.[a]	Assimilation relative to that of controls taken as 100.[a]			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O), relative to that of controls taken as 100.[a]
		Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
I	112	105	79	74	86
I	106	90	91	82	88
I	106	88	84	90	87
II	124	69	62	66	66
III	192	43	17	46	35
III	139	67	68	43	59
III	121	96	76	65	79
IV	180	49	28	36	37
IV	144	65	63	42	57
IV	170	43	20	49	37
IX	103	83	76	68	76
V	137	81	58	64	68
IX	169	52	17	46	38
VI	153	35	30	27	31
VI	131	68	41	48	52
IX	173	32	9	27	23
VII	187	45	23	30	33
VII	173	49	38	26	38
VIII	227	24	7	14	15

[a] The control plants were those grown with all their roots in the complete solution.

The more important facts established in the preceding experiments are as follows:

1. Depressions in growth and in assimilation of nutrients were roughly proportional to the incompleteness of the solutions afforded the roots, or, in other words, proportional to the extent the nutrients were restricted to separate portions of the roots.

2. Assimilation did not diminish with increasing subdivision of the roots among different solutions, unless the division entailed increased localization of the supply of the various nutrients.

3. The more growth and assimilation were depressed by division of the roots among incomplete solutions the higher was the ratio of root growth to top growth. However, the relation between diminution in assimilation and increase in the root to top ratio was not quantitatively proportional.

4. When different portions of the roots were supplied with different nutrient solutions, the roots in the more complete solutions generally made the greater growth and had a more bushy habit of growth. In the solutions lacking two elements the main roots were longer than in the more complete solutions, the lateral roots were fewer, and the laterals were farther apart on the main root.

5. When the roots were divided between solutions that were lacking in the same number of nutrients, root growth was greatest in the solution containing nitrogen.

6. The relative, as well as the absolute, root growth made in any solution, however, depended on the character of the solution in which the remainder of the roots were growing.

7. When the roots were divided among three incomplete solutions, each of which lacked either one or two of the elements, nitrogen, phosphorus, and potassium, the amount of nitrogen assimilated approached the normal assimilation of nitrogen—that is, the assimilation of plants with all their roots in a complete solution—considerably nearer than the amount of potassium assimilated approached the normal assimilation of potassium; also, potassium was assimilated to a very slightly nearer normal extent than phosphorus. This fact doubtless would not hold for all plants or for all stages of growth.

DISCUSSION OF RESULTS

The rate at which nutrient ions are assimilated by the plant is doubtless dependent upon the rates of absorption, translocation within the plant, and utilization, or the rate at which the ions are built up into complex compounds. These three rates of absorption, translocation, and utilization are, of course, mutually dependent, a reduction in any one reducing the other two.

The inability of a plant to effect a maximum assimilation of an ion which is supplied to only a portion of the roots evidently is not due to the root cells being unable to absorb this ion with sufficient rapidity. Data presented in the previous paper showed that roots could increase their rate of absorption very markedly. When only one-fourth of a plant's roots were supplied with nitrates, these roots absorbed nitrogen 2.26 times as rapidly as the roots of plants which were completely supplied with nitrates.

The diminished assimilation of nutrients when the roots are divided between incomplete solutions is more probably due to a diminution in the rate at which the nutrients are translocated to the cells where they are utilized. Although it is not known exactly how the ions are translocated, a rough explanation can be given of how the transference of ions in the vegetative part of the plant would be slowed down by absorption of the different nutrients by separate roots.

When, for instance, the nitrogen, phosphorus, and potassium are confined to separate roots (as in experiment VIII), there is an unusual transference of nitrogen to the roots in the phosphorus and potassium solutions, and an extraordinary transference of phosphorus to the roots in the nitrogen and potassium solutions, etc. The extra work of this unusual transference of nutrients, however, is hardly sufficient to account for the diminished assimilation.

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Probably the chief inhibition to translocation arises from the fact that nitrogen, phosphorus, and potassium are more or less scattered, as it were, in different parts of the plant, as a result of having been absorbed by different roots. Doubtless they are, for the most part, in different fibrovascular bundles and must be translocated by separate paths, instead of all together, to the cells where they are to be utilized. A cell, for example, which is adjacent to a fibrovascular bundle that emanates from a root in the phosphorus solution can secure phosphorus at once, but the nitrogen and potassium have to be transported from other centers in the plant. No more phosphorus can be assimilated by this cell until the nitrogen and potassium are secured. Under normal conditions the three elements would be obtained from the same fibrovascular bundle and they would be assimilated more quickly.

The foregoing suggestion concerning the manner in which translocation and assimilation may be depressed by a division of the roots between incomplete solutions seems to explain facts 1 and 2 of the summary given on page 568. The view that diminished assimilation is due to slow translocation rather than to a reduced power of absorption is also supported by the results of experiment VIII. In this experiment, where the assimilation was unusually low, the amounts of nitrogen and potassium in the roots were unusually large in proportion to the percentages in the tops.

It follows from the explanation of the way assimilation is depressed that when roots are in different incomplete nutrient solutions, slowness in the translocation or assimilation of one element reduces the rate of assimilation of other elements which are confined to other roots. This suggests a method, described below, for calculating the mean assimilation of those elements which are confined to certain roots, by making use of data presented by the authors in the previous paper.

This earlier paper contains a graph giving a curve which shows the assimilation of an element (relative to the normal) that would be attained when any fraction of the roots were deprived of one element, the remainder of the roots being in a complete solution. This curve, experimentally determined (reproduced in the present paper in fig. 1), agreed very closely with Mitscherlich's law of minimum.

While this curve is directly valid only for the condition where part of the roots are in a complete solution and part in a solution lacking one element, nevertheless, by utilizing values obtained from it, one can calculate fairly closely the mean of the amounts of nitrogen, phosphorus, and potassium that will be assimilated when the roots are in two or three different solutions each of which is lacking in one or more of these elements. It is only necessary to take from the curve the assimilations of nitrogen, phosphorous, and potassium (expressed as percentages of the normal assimilations) which correspond to the respective fractions of the roots supplied with these elements and then multiply the percentages

of assimilation together. The result will agree fairly well with the mean of the amounts of nitrogen, phosphorus, and potassium actually assimilated. In experiment VIII, for instance, 42.3 per cent of the roots were supplied with nitrogen, 27.7 per cent were supplied with phosphorus, and 30.1 per cent were supplied with potassium. Under the conditions for which the curve is valid, the assimilations corresponding to these fractions of the roots would be 67, 56, and 57 per cent, respectively. These percentages multiplied together give 21 per cent, and the actual

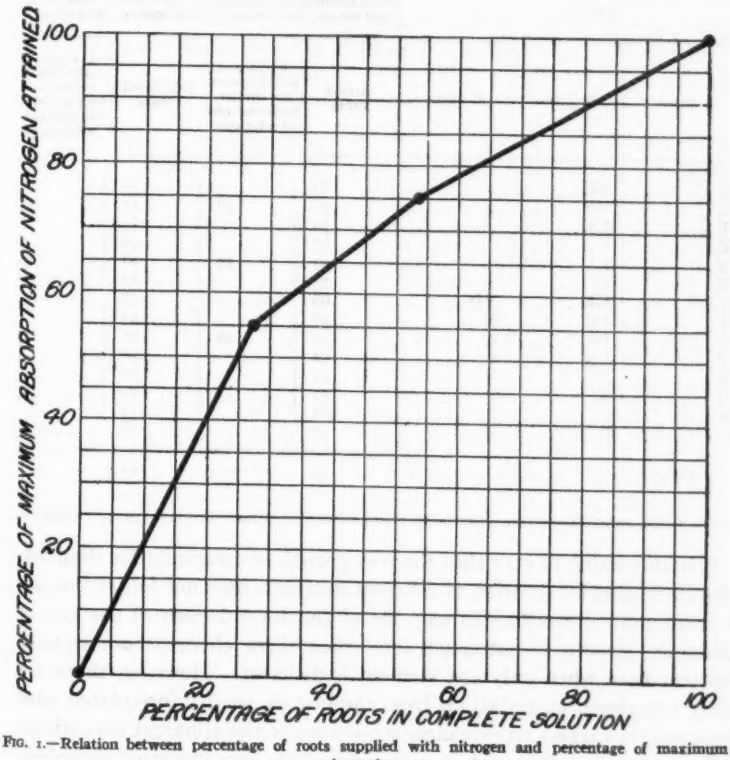

FIG. 1.—Relation between percentage of roots supplied with nitrogen and percentage of maximum absorption.

mean assimilation in this experiment was 15 per cent of that of the normal or control plants.

In Table XIX are given the actual and calculated values for the mean assimilations of nitrogen, phosphorous, and potassium in experiments where the roots were in incomplete solutions.

The agreement between the calculated and actual values is, for the most part, fairly close for work of this nature. The average values for a general type of combination of solutions, such as two solutions lacking

one element combined with one solution lacking one element, agree more closely than the values for any one combination of specific solutions. This is because the method of calculation does not take into account certain less important factors, such as the apparent greater need of the plant for nitrogen than for phosphorus or potassium.

TABLE XIX.—*Actual and calculated values of mean assimilations of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O)*

Solution.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to the normal taken as 100.[a]			
A flasks.	B flasks.	C flasks.	Actual value.	Average of actual values for similar combinations of solutions.	Calculated values.	Average of calculated values for similar combinations of solutions.
PK	NK		35	58	52	53
PK	NP		59		52	
NP	NK		79		54	
PK	N		37	44	36	40
NP	K		57		42	
NK	P		37		42	
PK	NK	NP	68	.....	57	.....
NK	PK	N	38	40	40	44
NP	PK	K	31		46	
NP	NK	P	52		46	
NK	P	N	23	31	30	32
PK	N	P	33		31	
NP	K	P	38		34	
N	P	K	15	.....	21	.....
Average			43	.....	42	.....

[a] The normal assimilation is that of the control plants grown with all their roots in the complete solution.

It is interesting to note that the way growth and assimilation diminish with increasing localization of different nutrients does not follow the law of minimum. According to any one of the formulations of the law of minimum, growth is not much less when three elements are equally deficient than when only one element is deficient. However, there are many experimental deviations from the law as usually formulated, and these results suggest an explanation for some of the apparent exceptions.

The fact that in this work the ratio of roots to tops increased as assimilation diminished may mean simply that a reduced assimilation of nutrients depresses the growth of roots less than it does the growth of tops. It may be, however, that a diminished assimilation of nutrients is directly stimulating to root growth. The growth of roots depends, of course, among other things, on the amount of organic material transported from the tops. Whether organic compounds are transferred to different parts of the leaves and stalks or to the roots may well be governed in part by the rate nutrient ions are utilized in the tops. When utilization is slow,

organic compounds may be transported to the roots; and when utilization is rapid, movement to the roots may be retarded. On such a basis, it might be said that slow utilization of nutrient ions, due either to a deficiency in the supply or to a reduced rate of translocation in the plant, would be stimulating to root growth. This, of course, applies to total root growth.

The relative amounts of root growth made in the different solutions (when the roots were divided) would depend on the relative needs of the plant for the nutrients present in the solutions, since the organic compounds would evidently be most quickly utilized in those roots which contained the greatest number of the essential ions.

REDUCTION IN THE STRENGTH OF THE MERCURIC-CHLORID SOLUTION USED FOR DISINFECTING SWEET POTATOES

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INTRODUCTION

The disinfection of seed sweet potatoes with mercuric-chlorid solution ($HgCl_2$) before bedding for the prevention of certain diseases has become a common practice since it was first recommended by Harter [2] in 1913. All investigators working on sweet potatoes recommend in general the same method of treatment, although they differ slightly on certain details of procedure. In fact it has never been satisfactorily determined how many bushels can be treated in a solution of 1 to 1,000 mercuric chlorid before it becomes so reduced in strength as to be no longer effective.

Some determinations have been made of the amount of mercuric chlorid removed from the solutions used for treating Irish potatoes, the results of which, so far as the writer is aware, have never been published.[3] However, the data thus obtained have influenced some investigators studying Irish potato diseases to recommend either that the solution be discarded after having been used three or four times or that a definite quantity of mercuric chlorid be added from time to time to bring the solution back to approximately its original strength.

Orton [4] states that the solution used for disinfecting Irish potatoes grows rapidly weaker, even losing as much as one-fourth of its strength during a single treatment. He recommends that this loss be offset by the addition of 1 ounce of mercuric chlorid to each barrel of solution after one batch of potatoes has been treated. It is suggested that after repeating this process three or four times the solution be discarded. This method of procedure for the treatment of Irish potatoes seems to be pretty generally agreed upon, since it is sanctioned by pathologists from several of the leading Irish potato growing States.

It has been assumed that Irish and sweet potatoes probably produce a similar change in the solution in which they are treated. Hence,

[1] The writer is indebted to Dr. L. L. Harter for valuable suggestions and criticisms during the progress of the work reported in this paper.

[2] HARTER, L. L. CONTROL OF THE BLACK-ROT AND STEM-ROT OF THE SWEET POTATO. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 114, p. 15–18. 1912.

[3] Since this article was submitted for publication results of a similar nature have been published. (BRANN, J. W., and VAUGHAN, R. E. POTATO SCAB. Wis. Agr Exp. Sta. Bul. 331, 27, p., 11 fig. 1921.)

[4] ORTON, W. A. SELECTION AND TREATMENT OF SEED POTATOES TO AVOID DISEASES. U. S. Dept. Agr. Bur. Plant Indus. C. T., and F. C. D. Circ. 3, 8 p., 2 fig. 1919.

recommendations have been made that the solution be discarded after 3 or 4 bushels of sweet potatoes were treated. Harter [1] states that the solution should not be used more than two or three times, since it loses its efficiency by repeated use. On the other hand, Taubenhaus [2] advises that the solution be used until exhausted.

The experiments reported in this paper were designed (1) to determine the rate of reduction in the strength of the mercuric-chlorid solution used for treating sweet potatoes and (2) to work out a method of procedure for growers whereby they might be reasonably sure of maintaining the solution at approximately its original strength with the least expenditure of time and money.

METHODS

The sweet potatoes used in these experiments were of the Yellow Jersey variety, grown on a sandy soil and stored in crates. The potatoes were sorted and placed in bushel hampers in which they were treated. In this manner the amount of dirt added to the solution was reduced to a minimum—that actually clinging to the potatoes. The solution was made up in a clean 50-gallon oak barrel by adding 4 ounces of mercuric chlorid previously dissolved in warm water to 32 gallons of water. Samples of the solution were taken for analysis both before being used and from time to time as indicated in the tables. In order to keep the solution as nearly as possible at its original strength, ¼ ounce of mercuric chlorid was added after each 10 bushels of potatoes were treated. In the experiments where the amount of mercuric chlorid removed by a specific substance, such as dirt, bags, hamper, etc., was being tested, the receptacle used for the solution was of such a nature that no reaction would take place between it and the chemical. In all such experiments, except where the hamper and the 30 pounds of potatoes were treated, a 2½-gallon porcelain bucket containing 2 gallons of solution was used, and in those two cases a 10-gallon glazed earthen jar with 7 gallons of solution was used.

The samples were analyzed the same day the treatments were made, with the exception of those in the experiments, the results of which are recorded in Table I. These were made the following day. All analyses were made by the volumetric method described by Jamieson.[3] In brief, the method consists of precipitating the mercury as mercury zinc thiocyanate, collecting the precipitate on a filter, and titrating it with a standard solution of potassium iodate (KIO_3) in the presence of strong hydrochloric acid, using chloroform as an indicator.

[1] HARTER, L. L. SWEET POTATO DISEASES. U. S. Dept. Agr. Farmers' Bul. 714, 26 p., 21 fig. 1916.

[2] TAUBENHAUS, J. J. FIELD DISEASES OF THE SWEET POTATO IN TEXAS. Tex. Agr. Exp. Sta. Bul. 249, 22 p., 34 fig. 1919.

[3] JAMIESON, George S. THE GRAVIMETRIC AND VOLUMETRIC DETERMINATION OF MERCURY PRECIPITATED AS MERCURY ZINC THIOCYANATE. *In* Jour. Indus. Engin. Chem., v. 11, no. 4, p. 296–297. 1919

Duplicate samples of 100 cc. each were placed in clean 250-cc. beakers, to which were added 25 cc. of the precipitating reagent, which consists of 39 gm. of ammonium thiocyanate and 29 gm. of zinc sulphate per liter. The solutions were vibrated by striking the sides of the beakers with a stirring rod to facilitate the separation of the crystals. After five minutes they were stirred rapidly for one minute and then allowed to stand for one hour or longer. Each solution was then filtered through a No. 42, 9-cm., chemically prepared Whatman's filter paper by the aid of gentle suction. The filter paper and precipitate were washed thoroughly with a solution made by adding 10 cc. of the thiocyanate reagent to 450 cc. of water. When the filter paper was thoroughly drained it was removed to a glass-stoppered titration bottle. To each bottle was then added a mixture consisting of 35 cc. of concentrated hydrochloric acid, 10 cc. of water, and 7 cc. of chloroform. This solution was then immediately titrated against a standard potassium-iodate solution containing 19.2191 gm. of potassium iodate per liter. The iodin liberated gave a brilliant red color to the chloroform, which changed to a pink and disappeared altogether when the end point was reached. The chloroform settled to the bottom of the bottle, and even in the presence of a considerable amount of dirt the end point was not obscured. Hence it was never necessary to filter the solutions to be tested.

In previous years attempts were made to carry out experiments of a similar nature, using the potassium-cyanid method for the determination of mercury. This method consists in titrating the mercuric-chlorid solution against a standard potassium-cyanid solution, using phenolphthalein as an indicator. In order to detect the end point, it was necessary to have a clear, colorless solution, which was often impracticable, especially since every attempt made to clear the solutions resulted in a decrease in the amount of mercury. The strength of a clean, standard solution of mercuric chlorid filtered through a single filter paper of various grades was reduced 1 to 3 per cent, and when two filter papers were used the concentration was lessened by as much as 6 per cent. In view of these facts a method had to be found in which filtering was not necessary. In one instance an attempt was made to determine the amount of mercury removed by a gunny sack by the use of potassium cyanid, but the analysis showed a larger amount of mercuric chlorid present in the solution after soaking the sack for 10 minutes than at the start. These results indicate that some substance present in the sack reacted with the potassium cyanid. No such inconsistencies were found in any of the analyses made by the Jamieson method. Some variation in the duplicate titrations occurred, but this was never more than one-half of 1 per cent and was usually less. The figures given in the tables are calculated from the averages of two or more titrations.

EXPERIMENTAL DATA

AMOUNT OF MERCURIC CHLORID REMOVED FROM THE SOLUTION DURING THE ORDINARY COMMERCIAL TREATMENT OF SEED SWEET POTATOES

In order to determine the amount of mercuric chlorid removed from the solution in which seed sweet potatoes were treated, two duplicate experiments were conducted, the results of which are recorded in Table I. A sample of the solution was taken in each case before any potatoes were added, and others were taken after each 5 bushels treated. After two 5-bushel lots were treated ¾ ounce of mercuric chlorid dissolved in water was added, the solution was stirred thoroughly, and another sample was taken. After four 5-bushel lots of potatoes were treated the solutions were made up to their original volume.

For the sake of convenience in presenting the data in the tables the 1-to-1,000 mercuric-chlorid solution is considered equal to 100 per cent, and all of the other concentrations are calculated on the same basis.

TABLE I.—*Percentage of mercuric chlorid removed from solution by treating 5 bushels of sweet potatoes in 32 gallons of solution for five minutes*

Number of bushels treated.	Amount of $HgCl_2$ added.	Percentage of $HgCl_2$ present in solution.		Percentage of $HgCl_2$ removed by each 5 bushels of potatoes treated.	
		Experiment 1.	Experiment 2.	Experiment 1.	Experiment 2.
	Ounces.				
0		96.6	95.6		
5		90.3	89.9	6.3	5.7
5		84.2	86.5	6.1	3.4
0	¾	103.1	102.3		
5		97.0	100.3	6.1	2.0
5		89.1	95.5	7.9	4.8
0	¾[a]	90.9	105.2		
5		87.1	103.4	3.8	1.8
Averages[b]				6.04	3.54

[a] Water was added to restore the solution to its original volume. This required 5 gallons in experiment 1 and 2½ gallons in experiment 2.
[b] Average for the two experiments=4.79 per cent.

An examination of Table I shows that in neither experiment was the original solution of a 1 to 1,000 strength. However, no attempt was made to measure the water accurately, but an effort was made to duplicate the method which a sweet-potato grower would probably use. The results of the analyses in Table I, experiment 1 (treatment 5 minutes), show that the first 5 bushels of potatoes treated reduced the strength of the solution 6.3 per cent, the second 5 bushels 6.1 per cent, the third 6.1 per cent, the fourth 7.9 per cent, and the fifth 3.8 per cent, making an average reduction of 6.04 per cent for each 5 bushels of potatoes treated. In experiment 2 (treatment 10 minutes) the first 5 bushels reduced the

strength of mercuric chlorid 5.7 per cent, the second 3.4 per cent, the third 2 per cent, the fourth 4.8 per cent, and the fifth 1.8 per cent, or an average reduction of 3.54 per cent. The potatoes used in the latter experiment had been employed in previous experiments of another nature, and some of them had had a considerable part of the dirt removed by handling or by being dipped into water. The potatoes used in the first experiment, however, had been selected for seed in the autumn, were stored in crates, and had not been handled until they were placed in the baskets in which they were treated. Nevertheless, data to be presented later will make it clear that a considerable amount of variation is to be expected.

In both experiments ¾ ounce of mercuric chlorid was added after each 10 bushels treated, in an endeavor to bring the solution up to its original strength. No data were at hand from which the correct amount of mercuric chlorid to be added could be calculated. It will be noted that in all but one case—namely, in the first experiment—after 20 bushels were treated, the amount of mercuric chlorid added was more than enough to return the solution to its original concentration.

Table I shows that within the limits of these experiments the addition of ¾ ounce of mercuric chlorid after 10 bushels of potatoes were treated sufficed to keep the disinfectant up to a strength where it was probably effective for the treatment of a total of 20 bushels without the addition of water. However, the thoroughness with which the potatoes are drained on removal from the barrel determines to a considerable extent the amount of water it is necessary to add. If reasonable care is exercised 20 bushels may be treated without the addition of water.

MERCURIC CHLORID REMOVED BY CLEAN SWEET POTATOES

The results obtained by the analyses of the solutions used for disinfecting sweet potatoes according to the method already described show that a considerable amount of the mercuric chlorid was removed. However, the results give no clue as to what was responsible for the change produced in the concentration of the solution. An attempt was next made to determine to what extent the potatoes, the dirt, and the container were responsible for the reduction in the strength of the mercuric chlorid.

The ability of potatoes, which were without doubt the most bulky substance added to the solution, to take up mercury was tested first. Six pounds of sweet potatoes from the storage house were washed free from dirt in running water, dried, and then immersed in 2 gallons of a solution of mercuric chlorid for 1½ hours, a sample being taken before the potatoes were added and again at various intervals, as indicated in Table II. The solutions of mercuric chlorid used in all of the following experiments were prepared separately.

TABLE II.—*Percentage of mercuric chlorid removed from solution by treating 6 pounds of washed sweet potatoes in 2 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.		Percentage of $HgCl_2$ removed from solution.	
	Experiment 1.	Experiment 2.	Experiment 1.	Experiment 2.
Control [a]	98.2	100.0	.....	.....
5 minutes	97.2	98.8	1.0	1.2
10 minutes	96.9	98.3	1.3	1.7
30 minutes	96.8	98.0	1.4	2.0
60 minutes	97.1	.....	1.1	.....
90 minutes	97.2	98.0	1.0	2.0

[a] Solution unused.

The control in Table II and in other tables to follow shows the concentration of the solution before the potatoes were added. In experiment 1 there was a decrease of 1 per cent in the amount of mercuric chlorid present after 5 minutes and a decrease of 1.3 per cent, 1.4 per cent, 1.1 per cent, and 1 per cent after 10, 30, 60, and 90 minutes, respectively. These results may seem inconsistent, but as a matter of fact the variations are well within the limits of experimental error, since a difference of 0.3 per cent is equivalent to only 0.05 cc. of potassium iodate. It is evident, then, that the potatoes removed approximately 1 per cent of the mercuric chlorid during the first 5 minutes and no appreciable amount thereafter, at least up to 1½ hours.

Experiment 2 was an exact duplicate of experiment 1, except that no sample was taken at the end of 60 minutes. Here there was a reduction in mercuric chlorid of 1.2 per cent after 5 minutes, 1.7 per cent after 10 minutes, and 2 per cent thereafter up to the end of the experiment. Again the greatest decrease occurred during the first 5 minutes, followed, however, by a further slight reduction during the next 5 minutes. The reduction of 0.3 per cent after 30 minutes is well within the limits of experimental error.

These two experiments show that the greatest reduction in the concentration of the mercuric chlorid took place during the first 5 minutes of treatment. However, it was thought desirable to conduct a similar experiment on a larger scale. Accordingly 30 pounds of washed potatoes were treated in 7 gallons of mercuric-chlorid solution, and samples were taken for analysis, the results of which are given in Table III.

The results show a reduction of 2.4 per cent in the amount of mercuric chlorid at the end of the first five minutes, the concentration remaining practically constant thereafter. These experiments show that the sweet potatoes removed some of the mercuric chlorid from the solution and that by far the largest part was taken out during the first five minutes.

TABLE III.—*Percentage of mercuric chlorid removed from solution by treating 30 pounds of washed sweet potatoes in 7 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	99.6	.....
5 minutes	97.2	2.4
10 minutes	97.4	2.2
30 minutes	97.2	2.4
60 minutes	97.2	2.4
90 minutes	97.0	2.6

[a] Solution unused.

A COMPARISON OF THE AMOUNT OF MERCURIC CHLORID REMOVED BY IRISH AND SWEET POTATOES

Since it was customary to recommend that the solution used for treating sweet potatoes be discarded after 3 or 4 bushels had been treated on the assumption that sweet potatoes would remove from the solution about the same amount of mercuric chlorid as Irish potatoes, a comparative test with Irish potatoes was made. Six pounds of Irish potatoes of the Burbank variety were washed and treated, a sample being taken before disinfecting the potatoes and at stated intervals thereafter, as shown in Table IV.

TABLE IV.—*Percentage of mercuric chlorid removed from solution by treating 6 pounds of washed Irish potatoes in 2 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	97.2	.....
5 minutes	96.4	0.8
10 minutes	96.4	.8
30 minutes	96.4	.8
60 minutes	96.4	.8
90 minutes	96.0	1.2

[a] Solution unused.

The results given in Table IV show that 6 pounds of washed Irish potatoes reduced the strength of the mercuric-chlorid solution to a slightly less extent than did an equal weight of sweet potatoes (Table II). They show further that Irish potatoes, like sweet potatoes, remove most of the mercuric chlorid during the first five minutes of treatment.

OTHER MATERIALS INVOLVED IN THE REMOVAL OF MERCURIC CHLORID DIRT

For the purpose of determining the extent to which other materials associated with the treatment are responsible for taking up the mercuric chlorid from the solution, tests were made with dirt, sacks, a hamper, concrete, and a barrel. Three types of dirt were used. Sample 1 was composed of the sweepings from a sweet-potato storage house and consisted chiefly of soil and fine roots which had collected where crates had been moved about or potatoes picked over. This was run in duplicate. Sample 2 was obtained from a field on which rye had been grown during the previous autumn and spring at the United States Department of Agriculture Experimental Farm at Arlington, Va., and had just been plowed under a short time before the sample was taken. The soil was a dark loam and contained roots of rye and weeds. Sample 3 was a red clay subsoil almost or entirely devoid of humus, taken several feet below the surface. One pound of each sample was treated in 2 gallons of mercuric-chlorid solution, and the results are given in Table V.

TABLE V.—*Percentage of mercuric chlorid removed from solution by treating 1 pound of dirt in 2 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.				Percentage of $HgCl_2$ removed from solutions.			
	Soil sample 1.		Soil sample 2.	Soil sample 3.	Soil sample 1.		Soil sample 2.	Soil sample 3.
Control[a]	91.1	100.0	101.9	94.0	.....	.....	.....	.....
5 minutes	75.8	82.4	100.5	93.0	15.3	17.6	1.4	1.0
10 minutes	75.5	82.0	97.6	92.2	15.6	18.0	4.3	1.8
30 minutes	75.5	82.0	.....	.....	15.6	18.0	.....	.....
60 minutes	75.8	.....	.....	.....	15.3	.....	.....	.....
24 hours	63.6	68.2	.....	.....	27.5	31.8	.....	.....

[a] Solution unused.

The results presented in Table V show a considerable difference in the amount of mercuric chlorid removed by the three types of soil. The two sets of soil sample 1 removed 15.3 and 17.6 per cent, respectively, during the first 5 minutes, after which there was little or no change up to and including 60 minutes. At the end of 24 hours there was a decided decrease in the strength of the solution. There was some variation in the changes produced by this dirt in the two trials, but in general the results are similar. However, a decided difference is apparent in the results of the experiment with soil sample 2. After 5 minutes only 1.4 per cent of the mercuric chlorid was removed, as compared with 15.3 per cent and 17.6 per cent with soil sample 1. Another increase to 4.3 per cent took place in the next 5 minutes, while in these cases the amount of mercuric chlorid seemed to remain constant for some time after the first 5 minutes.

Soil sample 3 behaved in much the same manner as sample 2, removing 1 per cent during the first 5 minutes and 1.8 per cent at the end of 10 minutes. It seems probable that the variation in the amount of vegetable matter present in the soils was largely responsible for the differences in the amount of mercuric chlorid removed, although other factors may have been involved. Soil adhering to the potatoes or that present in the containers is therefore responsible for the removal of a considerable part of the mercury. In view of these facts the extent to which a solution should be used depends somewhat upon the amount of soil and roots adhering to the potatoes.

SACKS

The amount of mercuric chlorid removed by two sacks treated in 2 gallons of solution was next determined. One was a 2-bushel cotton grain sack of the ordinary type, and the other a gunny sack of about equal capacity which had contained a commercial stock feed. The grain sack was washed and dried before being treated. The gunny sack was turned inside out and shaken thoroughly to free it as nearly as possible of adhering materials.

An examination of Table VI shows that a grain sack and a gunny sack remove a considerable amount of mercuric chlorid even during a 5-minute treatment and an increasingly larger amount as time goes on. If the rate at which a sack will remove the mercuric chlorid is proportional to the amount of solution in which it is treated, then the amount of mercuric chlorid removed by the bag from about 32 gallons would be something like 1 per cent for each treatment. Since the sacks continued to remove the mercury for at least 24 hours, they alone would be responsible for the removal of enough of the disinfectant to interfere seriously with the effectiveness of the subsequent treatments. It would therefore seem unsafe to use sacks as containers in the treatment of sweet potatoes.

TABLE VI.—*Percentage of mercuric chlorid removed from solution by treating sacks in 2 gallons of solution.*

Length of treatment.	Percentage of $HgCl_2$ present in solution.		Percentage of $HgCl_2$ removed from solution.	
	Grain sack.	Gunny sack.	Grain sack.	Gunny sack.
Control [a]	94.6	101.5	.....	.....
5 minutes	84.5	91.2	10.1	10.3
10 minutes	77.9	87.3	16.7	14.2
30 minutes	72.7	82.5	21.9	19.0
60 minutes	70.4	78.6	24.2	22.9
4 hours	65.4	.....	29.2	.....
5½ hours	.....	65.5	.....	36.0
24 hours	54.8	10.0	39.8	91.5

[a] Solution unused.

HAMPER

Sweet potatoes are often stored in bushel hampers, and it is sometimes convenient to use these as containers in making the treatments. Table VII gives the results obtained by treating a hamper in 7 gallons of solution contained in a 10-gallon stone jar.

TABLE VII.—*Percentage of mercuric chlorid removed from solution by treating a bushel hamper in 7 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	97.3	.....
5 minutes	96.0	1.3
10 minutes	95.5	1.8
30 minutes	92.1	5.2
60 minutes	91.8	5.5
24 hours	68.8	28.5

[a] Solution unused.

An examination of Table VII shows that the hamper likewise removed some of the mercury, although a proportionally much smaller amount than the sacks. Nevertheless, it is to a certain extent responsible for reducing the strength of the mercuric-chlorid solution in the commercial treatments.

CONCRETE

Farmers sometimes use concrete tanks instead of barrels as containers for the disinfectant. In order to learn whether concrete will produce any change in the concentration of the mercuric-chlorid solution, the following experiment was conducted. Two concrete blocks, having a combined total area of 215 square inches and a weight of 9 pounds, with smooth surfaces except at one end, were immersed in 2 gallons of solution. The changes in the concentration of the solution are shown in Table VIII.

TABLE VIII.—*Percentage of mercuric chlorid removed from solution by treating concrete blocks in 2 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	94.2	.....
5 minutes	94.0	0.2
10 minutes	94.0	.2
2 hours	90.7	3.5
24 hours	84.4	9.8

[a] Solution unused.

These figures show that practically no mercuric chlorid was removed from the solution during the first 10 minutes. However, in 2 hours the strength was reduced 3.5 per cent, and in 24 hours 9.8 per cent. These results demonstrate that although concrete does not remove the mercuric chlorid as rapidly as some of the other materials used, yet it does cause reduction in strength.

BARREL

Table IX gives the results of analyses of samples taken from a solution made up in a clean oak barrel. These results show that no appreciable loss of mercury could be detected up to the second hour, when 1.5 per cent had been removed. After 24 hours the strength of the solution was reduced 9.7 per cent. Obviously the barrel also exerts an influence upon the strength of the solution.

TABLE IX.—*Percentage of mercuric chlorid removed from solution by a clean oak barrel*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	100.1	.....
10 minutes	100.5	0
30 minutes	100.1	0
60 minutes	100.1	0
2 hours	98.6	1.5
24 hours	90.4	9.7

[a] Solution unused.

GENERAL DISCUSSION

From a survey of the tables it is evident that several factors may be responsible for the removal of mercuric chlorid from solutions used for treating sweet potatoes. No doubt the potatoes themselves remove a major portion of it, but other materials which come in contact with the solution, such as the sacks, hamper, soil, barrel, or concrete, also reduce the concentration of the solution. In the two experiments of which the results are recorded in Table I, the method of treating the sweet potatoes in common use among growers was used. Here potatoes, dirt, hamper, and barrel were all present, and the average amount of mercuric chlorid removed by them was approximately 1 per cent for each bushel treated. There was considerable variation in the amount removed by each 5 bushels treated, probably due at least in part to the amount of dirt clinging to the potatoes and to the differences in their size. By the addition of ¾ ounce of mercuric chlorid after each 10 bushels of potatoes treated the solution was kept near enough to its original strength for all practical purposes. However, ¾ ounce of mercuric chlorid in most cases was a little more than enough to restore the solution to its original

strength. Had the experiment been continued long enough it is possible that the strength of the solution would have been increased sufficiently to injure the potatoes, as sweet potatoes are very susceptible to mercuric-chlorid injury. It was found that 6 pounds of washed sweet potatoes removed slightly more than 1 per cent of the mercuric chlorid from 2 gallons of the solution. A pound of dirt in 2 gallons of solution removed from 1.8 to 18 per cent of the mercuric chlorid, the variation being due to the type of soil used. Aside from the potatoes, dirt is probably responsible for a greater change in the strength of the solution than any other single substance. However, sacks used as containers reduced the concentration very rapidly, a gunny and a grain sack in 2 gallons of solution decreasing the strength 16.7 and 14.2 per cent, respectively, in 10 minutes. On the other hand, a bushel hamper removed the mercury less rapidly. In view of these facts it would seem best whenever possible to use hampers or other wooden containers for the treatment. However, if it is necessary to use sacks the amount of mercuric chlorid added from time to time should be slightly increased. The oak barrel as well as the concrete blocks weakened the strength of the solution.

The foregoing facts show that the factors involved in disinfecting sweet potatoes are so many and varied that it is unwise to make any sweeping generalizations. However, if it is assumed that the average of approximately 5 per cent is removed by each 5 bushels of potatoes reasonably free from soil and treated in hampers in a clean barrel containing 32 gallons of solution, then the following recommendations may be made: After each 10 bushels of potatoes treated, add from $\frac{2}{5}$ to $\frac{1}{2}$ ounce of mercuric chlorid and add water to make the solution up to its original volume. It is further recommended that the solution be discarded after the treatment of 50 bushels.

Since the dirt and other foreign matter remove some of the mercuric chlorid, it is important that the barrel be thoroughly cleaned out before a fresh solution is made up. It is impossible to state that a given number of potatoes under known conditions will remove a definite amount of mercuric chlorid. The results of experiments 1 and 2 given in Table II illustrate this point, since in both cases 6 pounds of washed sweet potatoes of the same variety and from the same source were treated in 2 gallons of solution and in the former case 1 per cent and in the latter 2 per cent of the mercuric chlorid was removed in 1½ hours. No doubt the surface area of the potatoes governs to a certain extent the quantity removed. It is impossible to know the type or quantity of dirt and refuse that will find its way into the solution, and these are both important in reducing the concentration. All of these facts show how unreliable any sweeping generalizations may be. Nevertheless, there is need for some definite method of procedure in treating

sweet potatoes; and in view of the data presented in Table I, the writer feels that the recommendations given are more reliable than any heretofore proposed.

SUMMARY

(1) A bushel of sweet potatoes, when treated in 32 gallons of a 1 to 1,000 mercuric-chlorid solution in the manner generally followed by farmers, reduces the strength of the solution approximately 1 per cent.

(2) The decrease in the strength of the mercuric-chlorid solution is due in part to the potatoes themselves.

(3) The dirt and fibrous roots as well as the containers of both the potatoes and the solution also remove a certain amount of the disinfectant.

(4) Washed sweet potatoes and Irish potatoes remove approximately the same amount of mercuric chlorid from the solution.

(5) The addition of from ⅖ to ½ ounce of mercuric chlorid and sufficient water to make the solution up to its original volume after each 10 bushels of sweet potatoes treated will maintain the solution near enough to its original strength for all practical purposes for the treatment of 50 bushels of sweet potatoes.

CATALASE, HYDROGEN-ION CONCENTRATION, AND GROWTH IN THE POTATO WART DISEASE

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An attempt was made by one of the authors [1] to determine the relative importance of the physiological factors of the hydrogen-ion concentration, catalase, oxidase activity, and osmotic concentrations which are correlated with growth. Determinations were made upon tissues in which overgrowth was induced by inoculation with *Bacterium tumefaciens* Sm. and T. and as a result of injury from freezing.[2] In both of these cases the production of overgrowth was attended by an increase in catalase activity and decrease in hydrogen-ion concentration. It was shown to be improbable that overgrowths in tissues infected with *Bact. tumefaciens* were produced by differences in osmotic concentration brought about by the organism, since no considerable difference in osmotic concentration between diseased and healthy tissue could be found. In the mosaic disease of tobacco, in which a decreased growth is shown in the palisade cells of the diseased areas, there was a corresponding decrease in catalase accompanied by increased hydrogen-ion concentration of the expressed juice.[3] These cases would lead one to believe that increase or decrease in hydrogen-ion concentration brought about inverse changes in catalase, since it is known that catalase deteriorates rapidly in acid solutions and remains constant at acidities between P_H7 and P_H8. Boas [4] reports that the acidity of the cell sap of potatoes affected by leafroll is less than that of healthy plants; but whereas diseased plants show an increase in catalase content in some cases, this relation did not hold generally.

An idea of the importance of the factors correlated with the production of overgrowths can be obtained only by a study of conditions attending their production by various means or by different types of organisms. Excellent material for this work, in which there is a marked growth response on infection of the tissue, was found in the wart disease of the potato (*Solanum tuberosum L.*) casued by *Chrysophlyctis endobiotica* Schilb. These overgrowths are very striking in appearance and size,

[1] HARVEY, R. B. RELATION OF CATALASE, OXIDASE, AND H+ CONCENTRATION TO THE FORMATION OF OVERGROWTHS. *In* Amer. Jour. Bot., v. 7, no. 5, p. 211–213. 1920.

[2] ——— HARDENING PROCESS IN PLANTS AND DEVELOPMENTS FROM FROST INJURY. *In* Jour. Agr. Research, v. 15, no. 2, p. 83–112, 3 fig., pl. 7–11 and A (col.). 1918. Literature cited, p. 108–111.

[3] ——— HYDROGEN ION CHANGES IN THE MOSAIC DISEASE OF TOBACCO PLANTS AND THEIR RELATION TO CATALASE. *In* Jour. Biol. Chem., v. 42, no. 3, p. 397–400. 1920.

[4] BOAS, Friedrich. BEITRÄGE ZUR KENNTNIS DES KARTOFFELABBAUES. *In* Ztschr. Pflanzenkrank., Bd. 29, Heft 5/6, p. 171–176. 1919. Literaturverzeichnis, p. 176.

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frequently equaling or even exceeding the normal size of the organ which they replace, and they develop very rapidly—much more so than bacterial tumors. For instance, the earliest wart infections at Freeland, Pa., this year were found about July 13, and they were then not larger than 1 to 2 cm. in diameter. By the last of July warts 5 cm. or more in diameter were abundant, and some could be found even as much as 10 cm. in diameter and weighing 200 gm. Evidently growth is exceedingly rapid in the infected tissues, and this disease affords excellent material for a separation of the physiological factors affecting growth.

The hydrogen-ion determinations were made by the potentiometric method, using a bubbling electrode. The tests were run in pairs with wart and healthy tuber tissue collected from the same plant while in full vegetative activity and taken directly to the laboratory for testing. The material was ground in a food chopper and the juice pressed out through a cloth sack. No effort was made to use always uniform pressure with the different samples, because the hydrogen-ion concentration of an expressed juice depends not upon the total concentration of buffer substances present but upon the ratio of their concentrations. The samples were tested without dilution. It was observed that oxidase activity was much greater in wart tissue, judging by the very dark color which the expressed juice acquired as compared with the light color of healthy juice.

TABLE I.—*Hydrogen-ion concentration in healthy and wart tissue from the same plant*

Variety.	Healthy.		Wart.	
	P_H.	C_{H^+}.	P_H.	C_{H^+}.
Prince Henry	6.17	6.7×10^{-7}	5.96	1.09×10^{-6}
Ehnola	6.36	4.3×10^{-7}	Immune.	Immune.
Irish Cobbler	6.49	3.2×10^{-7}	Immune.	Immune.
Rural New Yorker	6.45	3.5×10^{-7}	6.11	7.76×10^{-7}
Do	6.39	4.07×10^{-7}	6.05	8.99×10^{-7}
Up-to-Date	6.35	4.47×10^{-7}	5.99	1.02×10^{-6}
Edzell Blue	6.50	3.16×10^{-7}	Immune.	Immune.
Seedling 39168	6.67	2.14×10^{-7}	Immune.	Immune.
Seedling 38816	6.65	2.24×10^{-7}	6.09	8.13×10^{-7}
McCormick	6.49	3.24×10^{-7}	Immune.	Immune.
Carman	6.57	2.69×10^{-7}	6.01	9.77×10^{-7}
Rose No. 4	6.48	3.31×10^{-7}	Immune.	Immune.
Portuguese Purple	6.65	2.34×10^{-7}	5.90	1.26×10^{-6}
Early Prospect	6.46	3.47×10^{-7}	6.03	9.33×10^{-7}
Average	6.49	3.48×10^{-7}	6.00	9.71×10^{-7}

The catalase determinations were made on diseased and healthy tissue from the same plant, using fresh material. The Van Slyke amino-nitrogen apparatus was used.[1] All determinations were made by using

[1] HARVEY, R. B. RELATION OF CATALASE, OXIDASE, AND H+ CONCENTRATION TO THE FORMATION OF OVERGROWTHS. *In* Amer. Jour. Bot., v. 7, no. 5, p. 211–213. 1920.

fresh 12-volume hydrogen peroxid (Dioxogen) previously neutralized with calcium carbonate ($CaCO_3$). The amounts shown are cubic centimeters of O_2 evolved in 5 minutes of slow and approximately constant shaking. In most cases 50 gm. of tissue was ground with quartz and excess calcium carbonate in a mortar and made up to 1,000 cc. with water. In a few cases different amounts of tissue were used, but corrections have been made in the totals. The temperature during the tests could not be held constant, but the fluctuation was less than 2° C. for controls.

TABLE II.—*Catalase activity in healthy and wart potato tissue from the same plants as measured by cubic centimeters of oxygen evolved from 10 cubic centimeters of hydrogen peroxid in 5 minutes* [a]

Variety.	Temperature.	Healthy.		Wart.	
		Number of tests.	Average cubic centimeters O_2.	Average cubic centimeters O_2.	Number of tests.
	°C.				
{Rural New Yorker	21	3	5.0	14.1	4
Do	21	3	5.9	.....	.....
Do	19 to 20	4	7.8	17.0	5
Do	20 to 21	4	9.2	17.9	9
Do	18.5	.....	.....	18.6	5
Do	19 to 20	.....	.....	22.4	7
Do	21.5	8	8.9	.....	.....
Do	20.5	4	5.0	.....	.....
Early Rose	21.0	1	7.4	.....	.....
{Woodbury's White Rose	19.5	.....	.....	11.3	4
Do	23.5	4	10.4	.....	.....
Do	22.5	.....	.....	18.3	4
{Prince Henry	19.2	.....	.....	11.7	5
Do	21.0	.....	.....	22.4	4
Do	22.2	4	6.4	.....	.....
Do	23.0	3	7.9	.....	.....
{Portuguese Purple	22.5	.....	.....	28.1	4
Do	21.7	5	10.5	.....	.....
Average	.....	.....	7.8	17.9	.....

[a] Results of determinations for tubers of one plant are inclosed in braces.

The tables show the effect of this parasite in producing acidity in the tissues it infects, the average acidity of healthy tissues expressed by P_H 6.49, becoming P_H 6.00 in the warts. In no case does the acidity of healthy tissue approach that of diseased tissue. This indicates that the organism will be found to be an acid producer when it is successfully cultivated on artificial media and gives also the range of acidity for its activity in the host tissue. Similarly, catalase activity is very much greater in the overgrowths than in normal tubers, the values being expressed respectively by 17.9 and 7.8 cc. of O_2 evolved. In no instance does the catalase activity of healthy tubers approach that of warts by nearer than 10 per

cent, and in most cases is much less. The data therefore show conclusively that a strong positive correlation exists between catalase activity and growth even though the increased acidity of the medium would tend to increase the rate of destruction of catalase.

From the several studies here summarized, in which decreased acidity has been shown to be correlated with production of overgrowths and high catalase activity in two instances [1] and increased acidity with overgrowth production in the present case, it must be concluded that such changes in acidity have little to do with catalase or overgrowth, unless it should be found that increasing or decreasing the acidity toward a certain value lying about P_H 6.00 favors growth in the tissue affected. Acidity changes overlapping this value have not yet been found, but in the three cases investigated changes of reaction toward this value have been associated with overgrowth production. Further data are needed for deciding this point. It is remarkable how close the value for wart tissue lies to P_H 6.00. It varies not more than 0.1 P_H from this value, although the acidities of different potato varieties vary as much as 0.5 P_H from each other.

It is evident from Table I that differences in the acidity of the potato varieties are not associated with resistance to the disease as shown by the production of overgrowths.

SUMMARY

A study was made of hydrogen-ion concentration and catalase activity in a new type of plant overgrowth, the wart disease of the Irish potato caused by *Chrysophlyctis endobiotica*.

The hydrogen-ion concentration of wart tissue is constantly greater than that of healthy tubers from the same plant, the values being represented by P_H 6.00 and P_H 6.49, respectively.

Catalase activity is much greater in the wart tissue, the values being represented by 17.9 cc. of O_2 for diseased and 7.8 cc. for healthy tissue.

Catalase activity is strongly correlated with growth in spite of the higher acidity of the proliferation. This differs from other types of plant overgrowths previously studied in which diminished acidity is correlated with increased catalase and growth activity.

Differences in acidity of the varieties are not associated with immunity to the disease.

[1] HARVEY, R. B. RELATION OF CATALASE, OXIDASE AND H+ CONCENTRATION TO THE FORMATION OF OVERGROWTHS. *In* Amer. Jour. Bot., v. 7, no. 5, p. 211–213. 1920.

—— HARDENING PROCESS IN PLANTS AND DEVELOPMENTS FROM FROST INJURY. *In* Jour. Agr. Research, v. 15, no. 2, p. 83–112, 3 fig., pl. 7–11 and A (col.). 1918. Literature cited, p. 108–111.

EFFECT OF CROWNGALL INOCULATIONS ON BRYOPHYLLUM

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In November, 1919, Michael Levine published a paper[1] in which he called in question some of my recent work on crowngall,[2] maintaining as the result of his own experiments: (1) That the shoots found in leafy crowngalls *originate from the tumor tissue* rather than from groups of normal totipotent cells disrupted and set growing by the growth of the tumor as I have maintained; and (2) that if my theory of embryomas is correct any totipotent tissue should be set growing—for example, dormant buds—yet in *Bryophyllum calycinum* Salisb. the crowngall organism (*Bacterium tumefaciens* Sm. and T.) *has no stimulating effect on the formation of shoots, but rather an inhibiting effect.*

I had never tried *Bryophyllum calycinum* for crowngall inoculation until after the appearance of Mr. Levine's paper; then it seemed worth while to make some experiments with it. From statements in his paper I thought the plant might react differently from tobaccoes, geraniums, and other plants which had given me numerous crowngalls containing abortive roots or shoots, but I have not found it to do so. In fact, working on the same plant and using the same microorganism (the one I originally sent to his colleague, Dr. Isaac Levin) I have obtained results quite like those obtained on other plants, as may be seen from the illustrations accompanying this paper.

In passing, it may be said that removal of leaves from the parent plant before inoculation is not good technic because they would then have

[1] LEVINE, Michael. STUDIES ON PLANT CANCERS. I. THE MECHANISM OF THE FORMATION OF THE LEAFY CROWN GALL. *In* Bul. Torrey Bot. Club, v. 46, no. 11, p. 447-452, pl. 17-18. 1919. Abstract *in* Exp. Sta. Rec., v. 43, no. 3, p. 242. 1920. The summary and conclusions of Mr. Levine's paper are as follows:

"1. *Bacterium tumefaciens* inoculated by pricks of a delicate needle into the marginal notches of a leaf of *Bryophyllum calycinum*, where totipotent cells are present, results in the formation of a crowngall as readily as in other plants used for inoculation but without leafy shoots.

"2. Inoculation of *Bacterium tumefaciens* into the tissue of a leaf of *B. calycinum* in the vicinity of a small bud causes the formation of a gall and interferes with the normal development of the bud or leafy shoot.

"3. Inoculation of *Bacterium tumefaciens* into the midvein of a young or old leaf detached from or attached to the mother plant results in the development of a large gall without the development of leafy shoots.

"4. Inoculation of *Bacterium tumefaciens* into the growing region of the stem of a young plant causes the development of the ordinary crowngall with the occasional and subsequent development of a leafy shoot.

"5. *Bacterium tumefaciens* does not cause the formation of leafy shoots in *Bryophyllum calycinum* but rather inhibits and retards their normal development, when inoculated into the totipotent cells which appear at the notches of the leaf."

[2] SMITH, Erwin F. CROWNGALL STUDIES SHOWING CHANGES IN PLANT STRUCTURES DUE TO A CHANGED STIMULUS. Preliminary paper. *In* Jour. Agr. Research, v. 6, no. 4, p. 179-182, pl. 18-23. 1916.

——— EMBRYOMAS IN PLANTS (PRODUCED BY BACTERIAL INOCULATIONS). *In* Bul. Johns Hopkins Hosp., v. 28, no. 319, p. 277-294, 1 fig., pl. 26-53 on 14. 1917.

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little food and a meager water supply and hence be under the worst possible conditions for developing tumors and especially tumors containing shoots, and because owing to this procedure one would not then be able to distinguish between the specific crowngall stimulus and the general stimulus of separation, which in Bryophyllum sets all the leaf buds growing.

Mr. Levine's most serious criticism is the statement that the shoots in crowngall develop from tumor cells. According to my observations the crowngall stimulus does not create totipotent cells out of tumor cells, but only sets growing those totipotent cells which already exist in the invaded tissues. The tumor cell is a disoriented degenerate cell, given over to a hasty vegetative growth. It is not an embryo cell, and I know of no evidence going to show that it can develop subsequently into normal tissues, organs, or the whole plant; on the contrary, it tends steadily toward decay. Moreover, since the tissues are not killed, what becomes of the bud when inoculations are made in the leaf axil if the numerous shoots which appear in various parts of the subsequently developing tumor (as for example in Plates 102 and 103) are not growths from dislodged fragments of the bud? Furthermore, when a deep crowngall develops under the normal cortex, the cortex is lifted up and grows with the growth of the tumor without being actually a part of the tumor tissue. Certainly its cells have normal orientation, function normally, are no part of the malignant tissue and have not originated from it, although they are borne on it, as in Plate 101. See also Bulletin of the Johns Hopkins Hospital, September, 1917, figures 70, 71, 72 (okra tumors), and many other figures which I have published.

Concerning the tissue of vessels and mature cells contained in the substance of crowngalls, one may call it *stroma* if he likes. Every tumor has a stroma (supporting tissues), and it is not likely that the stroma in plant tumors would be exactly like that in animal tumors, although the amount of stroma is extremely variable in the latter. The crowngall stroma is organized along with the tumor and forms an intimate part of it, but I can not think that it is necessarily developed *out of infected cells*. We shall not know positively, perhaps, until we are able to stain the bacteria in situ. As in malignant animal tumors, the crowngall stroma appears to me to be a growth of normal tissues (vessels and connective cells) stimulated by the presence of the abnormal cells, just as the stimulated roots and shoots are outgrowths of normal cells; yet the latter, being no part of the actual tumor tissue, have normally arranged tissues whereas the tracheids of a crowngall, being a more intimate part of its structure (stroma), are often contorted into the most bizarre forms by the growth of the tumor. A crowngall in its growth often surrounds normal cells just as a cancer may or bears them on its surface. The number of vessels in a crowngall depends not on the activity of the tumor so much as on the nature of the tissue invaded—that is, a tumor

originating in some extremely vascular tissue is itself very vascular, much more so than one originating in a nonvascular tissue, although the latter always contains some vessels (tracheids) just as any wound repair tissue does.

To return now to Bryophyllum, when suitable inoculations are made under a dormant bud—that is, immediately under a petiole—the developing tumor not only stimulates the bud to develop into shoots, as may be seen in Plate 101, but sometimes even causes, like the disease in the peach tree called the peach yellows, a secondary set of branches to appear on such shoots, as may be seen under the arrows in Plate 101. Here the tumor-stimulated shoots are the only axillary shoots on the plant. Often the tumor tissue subsequently invades the swollen base of such a shoot.

On the contrary, when the inoculations are made directly into the leaf axil—that is, into the dormant bud and into tissues immediately surrounding it—centers of infection and of active growth begin around the needle pricks, and these disrupt the bud in various directions, widely separating its fragments, as may be seen in thin sections under the microscope; and subsequently these fragments are just as certain to feel the tumor stimulus and to develop as is the whole bud in the previous case, only, the food supply and water supply being divided and limited by distortion and rupture of the vascular bundles, the result will be the development in the axillary tumor of several to many stunted shoots or mere buds, rather than one or two strong shoots. This is illustrated in Plates 102 and 103, which are back and front views of the same inoculated plant.

This stimulus, moreover, contrary to Mr. Levine, not only causes axillary buds of Bryophyllum to germinate, but also leaf-notch buds. To have made his test of any value (conclusion 2), Mr. Levine should have inoculated leaves while still on the plant and not after removal, when most of the leaf-notch buds will grow indiscriminately whether inoculated or not. Also they should have been young leaves. When done in the right way, the buds, stimulated by crowngall, develop, and no others, as may be seen in Plate 104, where out of about 400 dormant buds situated on the margins of the 26 leaves only those on 2 inoculated leaves have developed into shoots; and even here, if I had made my inoculations *only on well-developed leaves* some or all of my results might have been negative, as happened on one of the lower leaves of this same plant. That here the growth of the dormant buds into shoots is attributable to the inoculations in their vicinity and to nothing else is plain, because leaf-notch buds do not develop at all on undisturbed young shoots. In old plants they grow freely out of the leaf notches of old leaves which are still green and very firmly attached to the stem—that is, not pathological—but the order of their development is different—that is, they first appear on the basal leaves and gradually extend to

those farther up. In Plate 104 they have appeared on two leaves in the middle of a young shoot (uppermost leaves at time of their inoculation) *and on these only*. Many controls, and also observations extending over a period of several years, teach me that this unusual development of the leaf buds on an otherwise undisturbed young plant can be attributed only to the stimulus of the neighboring marginal tumors; and, consequently, the response of totipotent cells in *Bryophyllum calycinum* is not different from that of similar cells in other plants.

There remains to consider what will happen when the infected needle is thrust directly into leaf-notch buds rather than into their vicinity. Mr. Levine says it results in the formation of a crowngall without leafy shoots (conclusions 1 and 5). Plates 105 to 108 are a sufficient reply to this, although they represent only a small part of the results obtained. Here single needle thrusts were made directly into dormant leaf-notch buds (toward the top of the plant), usually on one side of the leaf only;[1] and in nearly every case shoots as well as tumors have developed, and some of the small, slow-growing tumors bore several shoots and roots (35 roots in Pl. 108). I had many cases like this where shoots developed only from the inoculated notches, other buds on the same leaves remaining dormant. Shoots taken from single inoculated leaf notches a month earlier than Plate 105 (which is reduced) are shown enlarged five times on Plate 106. Here the small tumors at their base can be seen very distinctly. It will be observed also that the neighboring uninoculated notches are free from shoots. Later both the shoots and the tumors became larger. On the small stimulated shoot in at least two cases (Pl. 107) the buds in its lower leaf axils (under the arrows) also pushed just as in the shoots of Plate 101 and others in that series.

Most of the crowngalls produced on midribs of *Bryophyllum calycinum* are free from leafy shoots. They are free, however, not for the reasons assigned, but because totipotent cells are rather rare in the midribs of this plant, which has sent most of them to the edges of the leaf. Only rarely, therefore, might one expect the infected needle to reach a group of such cells. That crowngalls containing shoots and roots may be produced by midrib inoculations on Bryophyllum is sufficiently evident from Plates 109 and 110.

Totipotent cells occur abundantly in the midribs of tobacco, tomato, begonia, and pelargonium, but it does not follow that they occur in the same abundance in the midribs of all plants, and especially not in those of a plant as peculiar as this one. Where we *know* beyond peradventure

[1] This was probably not done in the best way to induce a breaking up of the bud tissue, since the needle was not thrust from the margins inward but from some distance inside the margin outward through the leaf parenchyma, parallel to the leaf surface, until it was judged that the bud had been reached, a matter always in some doubt because of its invisibility and small size. Three distinct shoots are the most I have seen develop from such an inoculation, but undoubtedly the bud can be ruptured so as to produce a much larger number of shoots.

that there are totipotent cells—in the leaf axils and in the leaf notches of Bryophyllum—*there I have got the same results with crowngalls as on other plants.*

I undertook to test my conclusion as to the rarity of totipotent cells in the midrib of *Bryophyllum calycinum* in another way—that is, on 150 well-developed leaves by means of sand-bed experiments. The margins were trimmed away and after some days the leaves were removed from the plants and bedded on damp sand, with the undersurface down and petiole buried. Just before this was done the midribs were cut crosswise in from 4 to 12 places, depending on the size of the leaf. After six weeks they were removed and examined. Of these leaves, 27 per cent developed roots (and sometimes shoots) from the base of the petiole, showing that totipotent cells are of fairly frequent occurrence in that organ, and 10 shoots appeared on the margin of leaf blades in spite of the fact that they had been trimmed, but, with exception of two cuts on one leaf where incipient roots appeared, none whatever (either roots or shoots) appeared on the cut midribs, although the conditions were favorable and there were at least 1,000 opportunities. This tends to confirm the conclusions derived from the crowngall inoculations. I conclude, therefore, that shoots are rare in the midrib tumors for the sole reason that totipotent cells are rare in such places. Yet if organs are developed directly from tumor cells, as Mr. Levine maintains, then why in the presence of such an abundance of tumor cells in active growth are shoots and roots so rare in midrib tumors on Bryophyllum? These tumors grow as vigorously as axillary tumors on Bryophyllum or as midrib tumors on tobacco, and should we not expect to find shoots as common in the former as in the latter, if this hypothesis is correct?

The exact nature of the stimulus that sets the leaf-notch buds growing in crowngall inoculation may be left for discussion elsewhere. It is sufficient here to have established that, contrary to Mr. Levine's statements, a distinct crowngall stimulus exists for the dormant buds of Bryophyllum.

PLATE 101

Middle part of shoot of *Bryophyllum calycinum*, showing results of inoculation with *Bacterium tumefaciens* (hop strain) on March 10, 1920. The needle was set in horizontally just below the lower left petiole. As the tumor developed it twisted the leaf and shoot and caused the bud above it to grow. This axil was the only one on the plant that developed any shoots. Other plants not photographed behaved in the same way. The shoots show secondary branching (under the arrows) and enormous enlargement at the base. At *XX* leaves were broken off. Root anlage at *R*. Photographed June 17, 1920.

R

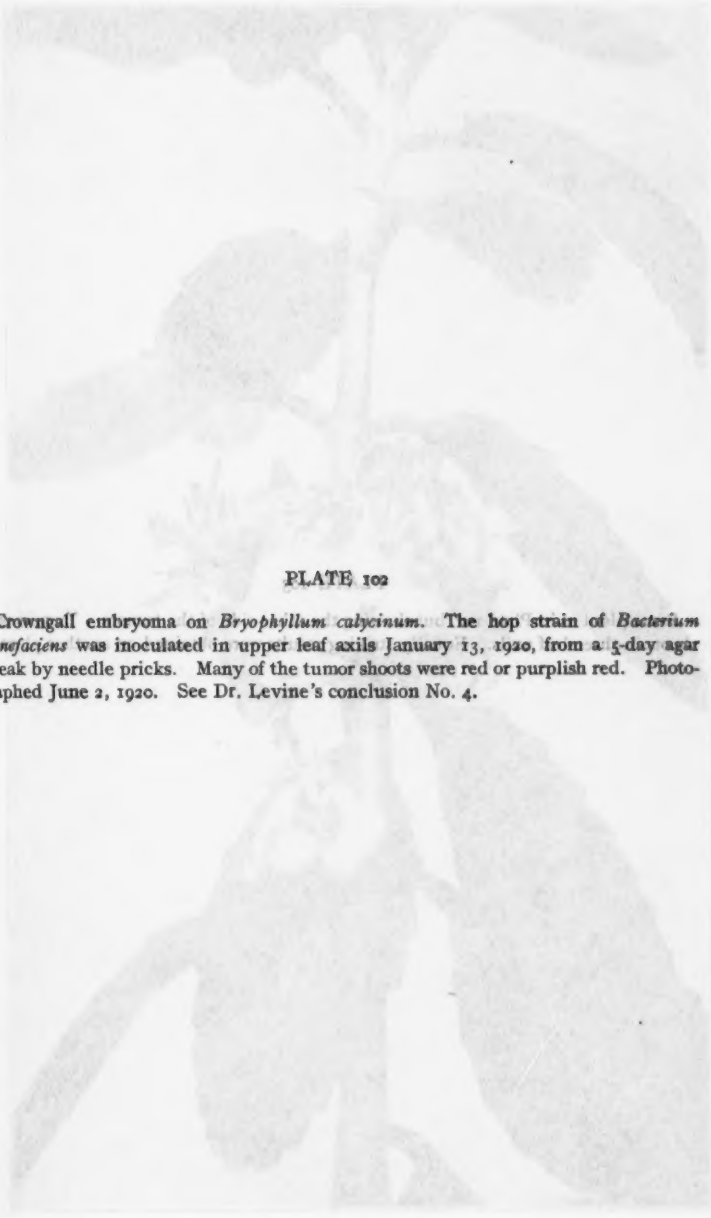


PLATE 102

Crowngall embryoma on *Bryophyllum calycinum*. The hop strain of *Bacterium tumefaciens* was inoculated in upper leaf axils January 13, 1920, from a 5-day agar streak by needle pricks. Many of the tumor shoots were red or purplish red. Photographed June 2, 1920. See Dr. Levine's conclusion No. 4.

PLATE 103

Same plant as in Plate 102, but showing the other side of the tumors. Tumors with shoots in three-leaf axils. Equally striking results were obtained on another plant.

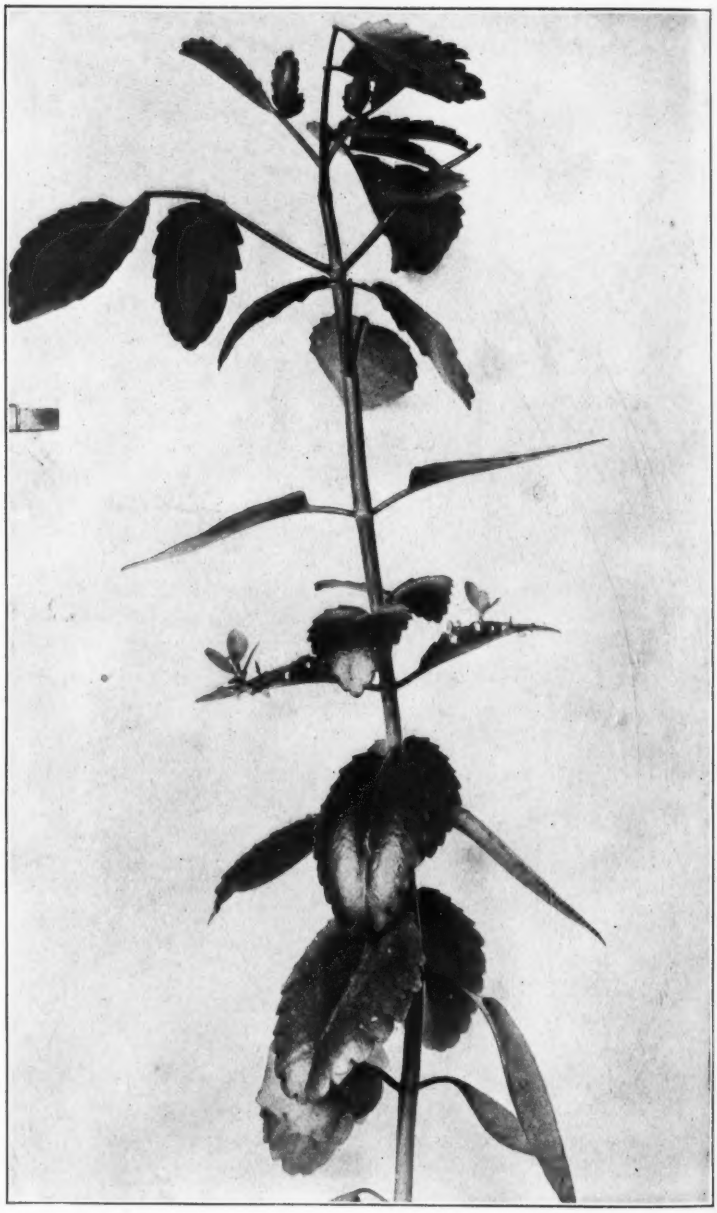


PLATE 104

Young shoot of *Bryophyllum calycinum*, showing crowngall stimulus—that is, a pair of leaves sending out shoots from their margins as a result of inoculating *Bacterium tumefaciens* (hop strain) in the vicinity of the leaf notches. This pair was plainly dwarfed. Inoculated January 13, 1920. Actual height of shoot 24 inches. Some of the upper leaflets were knocked off in bringing the plant from the hothouse to the laboratory to be photographed, but none of these bore any shoots. There were 12 shoots on the inoculated leaves. Photographed June 7, 1920.

48496—21——7

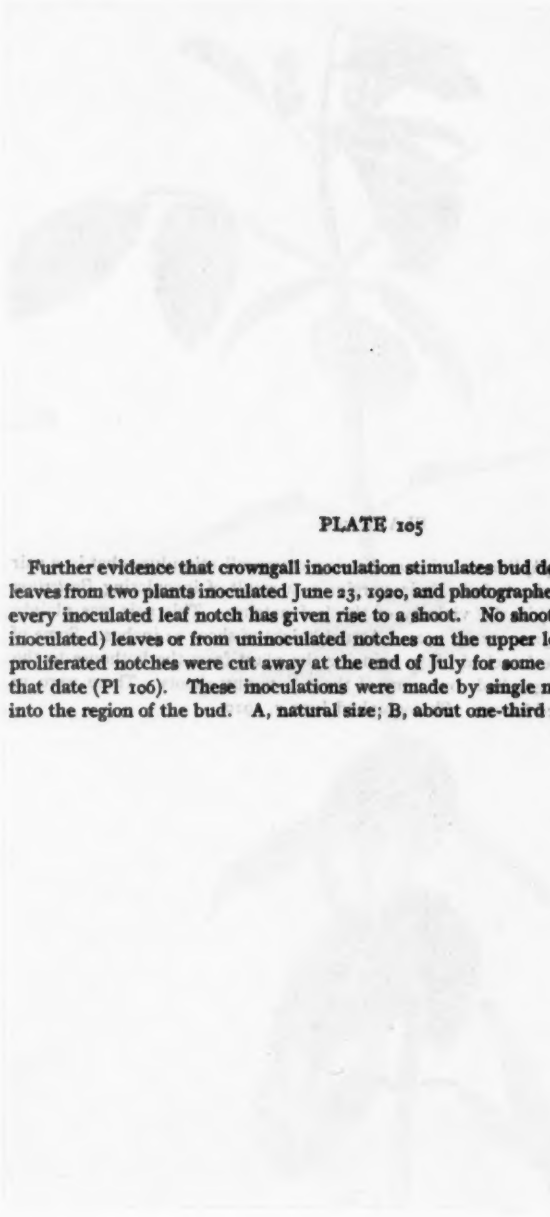


PLATE 105

Further evidence that crowngall inoculation stimulates bud development. Pairs of leaves from two plants inoculated June 23, 1920, and photographed August 31. Nearly every inoculated leaf notch has given rise to a shoot. No shoots from the lower (uninoculated) leaves or from uninoculated notches on the upper leaves. From *B* at *X* proliferated notches were cut away at the end of July for some of the photographs of that date (Pl 106). These inoculations were made by single needle thrusts directly into the region of the bud. A, natural size; B, about one-third natural size.

A
X
B

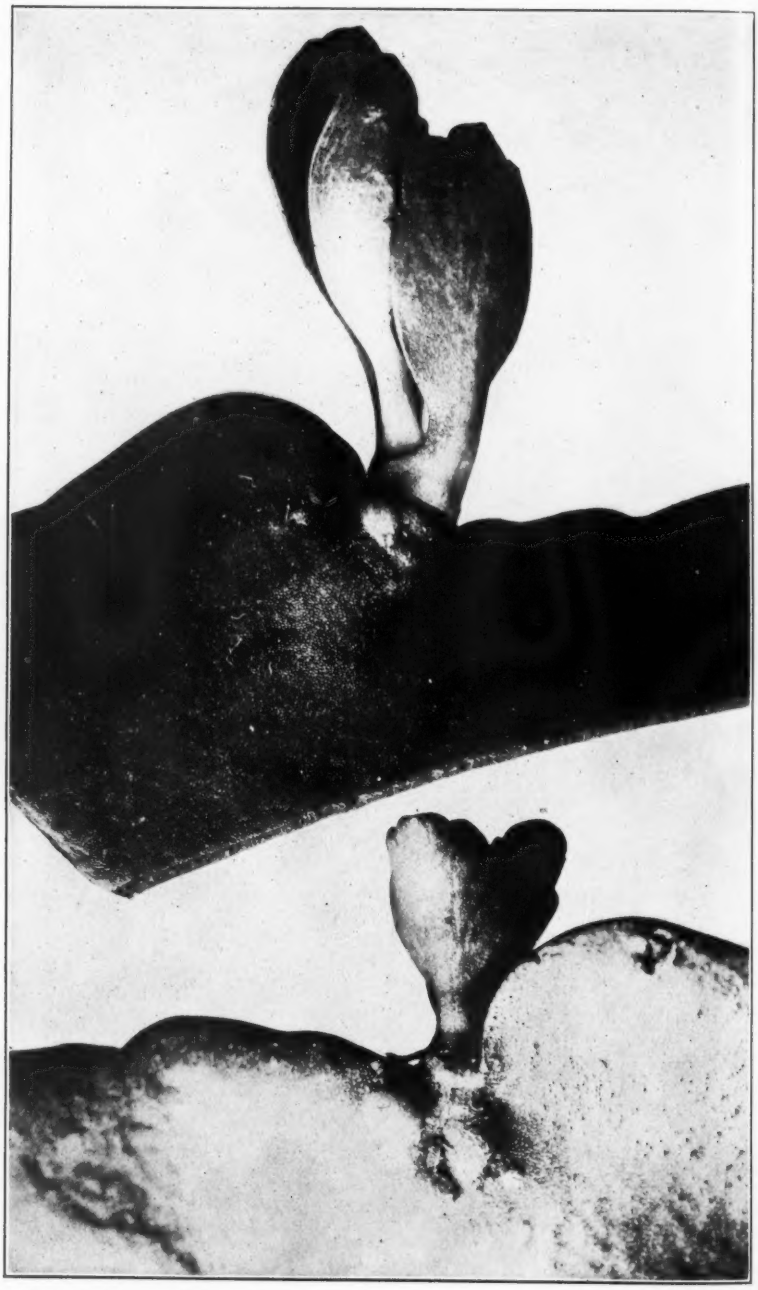
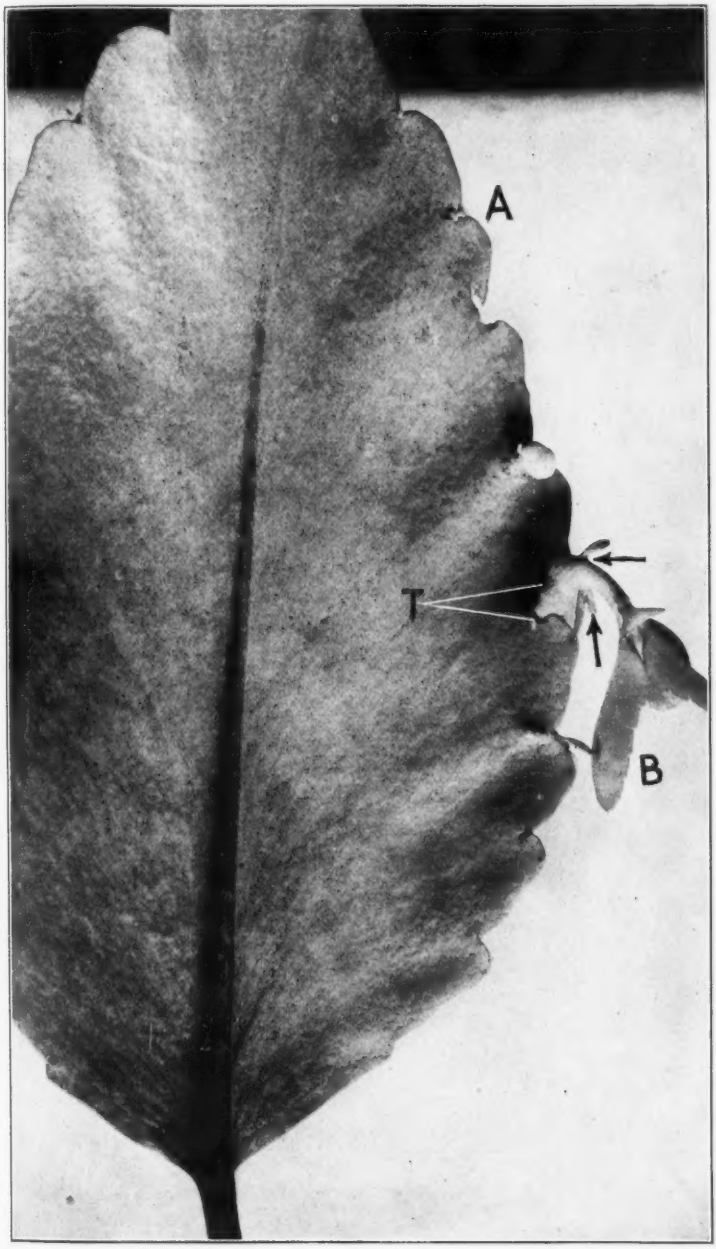


PLATE 106

Inoculated leaf notches cut away and photographed July 27, 1920, and enlarged to show the small tumors distinctly. Each was produced by a single needle prick introducing *Bacterium tumefaciens* (hop strain). Such shoots developed about a month after the inoculation and only from the inoculated notches. Most bore roots also, as in the lower shoot. × 5.

PLATE 107

Same series as Plate 108 but collected a month later. Only the five middle notches from *A* to *B* were inoculated. The arrows point to buds germinating in the lower leaf axils. The shoot is pushed out of its original location and is now borne on the top of the growing tumor, which, owing to the method of inoculation already described, began underneath it. The tumor proper lies at the base of the triangle *T*. Immediately above this is the swollen base of the shoot normal on its surface (structurally) but diseased within—that is, containing groups of disoriented tumor cells. Inoculated June 11, 1920. Photographed October 20. Natural size.


A
T
B

R
Rs
X

PLATE 108

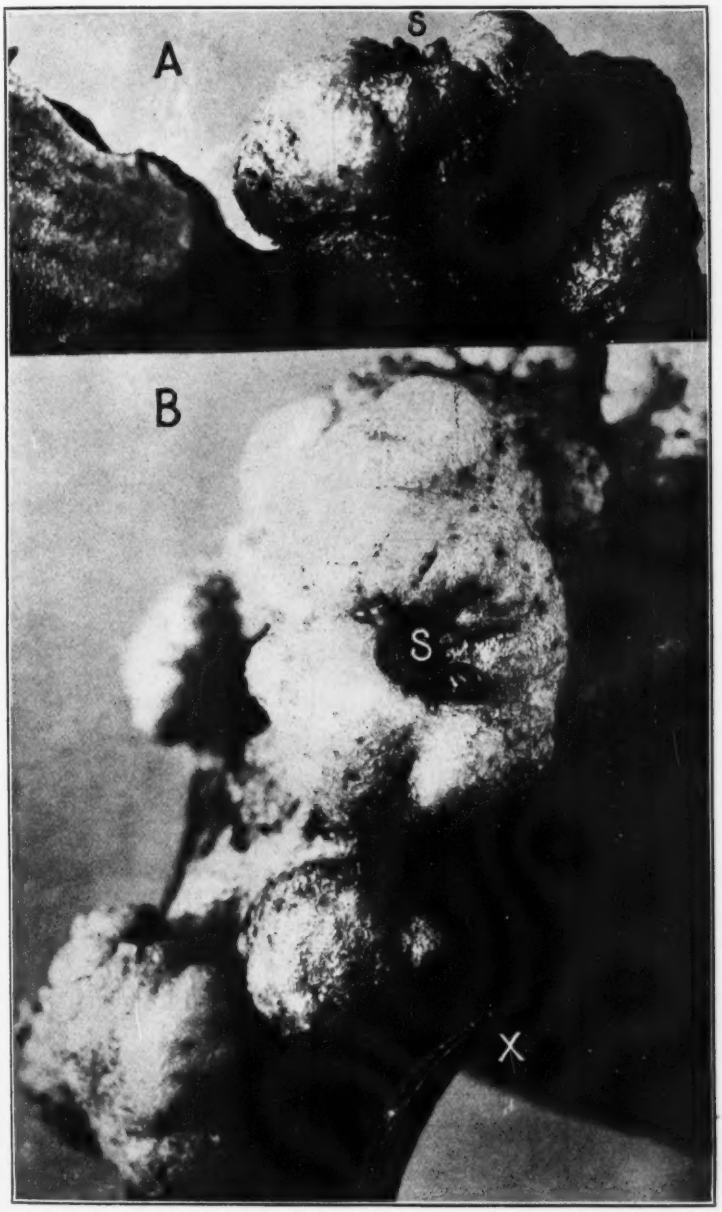
Crowngall from a leaf notch of *Bryophyllum calycinum*, showing 3 shoots and 35 roots. The arrow indicates the dividing line between naked tumor tissue (below) and the stretched, lifted up cortex (above) which contains normally oriented cells and surrounds disoriented tumor cells. *Rs*, roots; *R*, pushed up cortex where a root originating in the pericycle of the larger shoot has not yet reached the surface. At *X* another leaf notch was inoculated, but the tumor tissue (shown to be present by means of serial sections) is quiescent and the shoot is barely visible; yet judging from results obtained on the orange,[1] it might later have developed into a tumor as large as the upper one, especially if that had been removed. Inoculated June 11, 1920. Photographed September 22. ×5.

[1] SMITH, Erwin F. AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. Fig. 341. Philadelphia and London. 1920.

PLATE 109

A.—Side view of a pure white midrib tumor on *Bryophyllum calycinum*, showing at *S* two pale green shoots.

B.—Vertical view of A, showing the green shoots at either side of *S* and at *X* the twisted leaf blade in cross section. The leaf was bent double by growth of the tumor. × 5.


A
S
B
S
X

X
M

PLATE 110

Midrib crowngall on *Bryophyllum calycinum*, showing 5 roots developing from one of the tumor lobes at *X*. There were also 16 roots developing from other lobes under the arrow. Midrib at *M*. Midrib tumors on three other leaves in this series also showed roots. Inoculated June 23, 1920. Photographed November 18. × 5.

EFFECT OF THE LENGTH OF DAY ON SEEDLINGS OF ALFALFA VARIETIES AND THE POSSIBILITY OF UTILIZING THIS AS A PRACTICAL MEANS OF IDENTIFICATION

By R. A. OAKLEY, *Agronomist*, and H. L. WESTOVER, *Agronomist, Forage Crop Investigations, Bureau of Plant Industry, United States Department of Agriculture*

At Arlington Farm, Va., a few years ago the writers noted striking differences in the habits of growth of young seedlings of the commercial varieties of alfalfa produced from seed sown in the early fall. The differences in height and general appearance were so great that even a casual observer could easily distinguish certain of the varieties. Not having noted previously such a marked varietal difference in seedlings, the writers were led to the tentative conclusion that the time of sowing was, in some measure at least, responsible for the phenomenon. To test the validity of this conclusion, experiments were conducted the following year in which Peruvian,[1] Kansas,[2] Canadian variegated, Grimm, and a yellow-flowered alfalfa (*Medicago falcata* L.) were sown at Arlington Farm in the early fall. The same striking differences that had previously been observed were noted. However, May and June sowings at Arlington and also at North Ridgeville, Ohio,[3] showed no such marked varietal characteristics. This, in a measure, confirmed the conclusions the writers had reached and suggested further investigations, which were subsequently conducted.

POSSIBILITY OF DISTINGUISHING VARIETIES OF ALFALFA IN THE SEEDLING STAGE[4]

In addition to studying the effect on alfalfa seedlings of sowings made at different times of the year, it occurred to the writers that it might be possible in this connection to develop a practical method of identifying various alfalfas in a relatively short time after samples of seed are available. Such a method would be useful, indeed, especially in identifying the Grimm and Peruvian varieties. In fact, it was chiefly to find a means of identifying alfalfas quickly that detailed studies of seedlings were undertaken.

Partly because of availability of location and partly for the purpose of testing the effect of latitude on the growth of alfalfa seedlings,

[1] All references to Peruvian alfalfa in this publication are to the hairy strain.

[2] For the sake of convenience the term Kansas alfalfa refers to the regional strain of common alfalfa produced in Kansas.

[3] Experiments conducted cooperatively with the Ohio Agricultural Experiment Station at North Ridgeville, Ohio, about 3 miles north of Elyria.

[4] For the sake of convenience the term variety is used in its broad sense to mean regional strains as well as distinct horticultural varieties.

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sowings were made at North Ridgeville, Arlington Farm, and Bard, Calif. Early September sowings gave practically the same results at North Ridgeville as at Arlington Farm. The late fall and early winter sowings at Bard produced seedlings of much the same appearance as those from early September sowings at North Ridgeville and Arlington. This was also true of December sowings in the greenhouse at Washington. May and June sowings at Arlington and North Ridgeville showed practically no difference in the rapidity and habit of growth of the varieties tested. A range of field sowings from August 26 to September 16 at Arlington showed that five to six weeks of growing weather at that time of the year are required to produce plants of a satisfactory size for studying the varietal characters of the seedlings. The late August and early September sowings gave good results, but September 16, in this particular case, proved to be rather late.

From these tests it was evident that by sowing seed in late summer or early fall in the latitude of Arlington, and northward, it is easily possible to determine whether a given lot of alfalfa is of the Peruvian variety, the common variety (such as the various regional strains) or the variegated (such as the Grimm, Baltic, and Canadian variegated). From spring or summer sowings, especially in the North, the varietal differences exhibited are hardly great enough to be regarded as sufficient for the untrained person to recognize with certainty. Thus far in the investigations this method is limited in its application to field sowings in the fall in the North and to winter sowings in the extreme South. It is also limited to distinguishing between groups of alfalfas, such as the common or the variegated, rather than between the strains in these groups, as for example the Kansas-grown common strain and the Dakota-grown common strain or the Grimm, Baltic, and Canadian variegated. But the method makes it easily possible to distinguish between the Peruvian and the other alfalfas.

COMPARATIVE CHARACTERISTICS OF SEEDLINGS

The differences that were most noticeable in the seedlings resulting from fall sowings at Arlington were those of height, branching, and compactness. The Peruvian produced erect, sparsely branched seedlings of greater height than those of the other varieties. They were also more nearly uniform. The seedlings of the Kansas variety very closely resembled those of the Peruvian variety in uniformity and general habit of growth but were not nearly as tall and showed more of a tendency to send out basal branches. The Grimm seedlings were much shorter, more branched, and less erect than those of the Peruvian or Kansas alfalfas. They also showed more variation than these varieties. The seedlings of *Medicago falcata* were uniform, very short, and exhibited a rosette-like habit. The Grimm seedlings were nearly intermediate between the Kansas and *M. falcata*. The difference between the seedlings

of the several varieties is well illustrated in Plates 111 and 112. While there were some differences between the seedlings of the several varieties from May and June sowings, the Kansas and Grimm more nearly approached those of the Peruvian in height and very closely resembled each other. The Grimm seedlings did not take on the branching or rosette-like habit of growth so characteristic of seedlings from fall sowings. The spring seedlings of the Grimm were also much more uniform than the fall seedlings and closely resembled the Kansas and Peruvian in this characteristic. (Pl. 113, 114, 115.)

RELATION OF ALFALFA SEEDLINGS TO PHOTOPERIODISM

After a study of the behavior of the alfalfa seedlings from sowings made at different times of the year and at different latitudes it was concluded that the marked differences exhibited by seedlings of the several varieties from fall sowings at Arlington and North Ridgeville were due chiefly to the effect of cool weather. With no marked varietal differences from the May and June sowings, this conclusion seemed entirely justified. The significance of the fact that greenhouse tests made in December at Washington produced striking differences between the varieties was not appreciated by the writers at that time. Later, however, the results of Garner and Allard [1] from their investigations in the photoperiodism of plants caused the writers to suspect that the length of day might, to some extent, be involved in the phenomenon exhibited by the seedlings of the various alfalfas included in their studies. Consequently experiments were outlined and undertaken in the greenhouse on the Department of Agriculture grounds at Washington. On January 20 seed of Peruvian alfalfa, Kansas, Grimm, Turkestan, and a strain of *Medicago falcata* was sown in three flats. One flat was given the effect of the normal length of day, one was put in total darkness at 4 p. m. and left there until 9 a. m., and one had the length of day augmented by an electric light and reflector placed about 2 feet above it. The light was turned on a half hour before sundown each day and turned off at 11 p. m. The length of both the short and long day was fixed at what was regarded as extreme. The former was fixed largely as a matter of convenience to suit the working hours of the gardener and the latter to insure the effect of a considerably increased period of illumination. The day-night treatments apparently produced no effect on the germination of the seeds, but as is almost invariably the case the *M. falcata* seed germinated more slowly and unevenly than the others. The Grimm and Turkestan resembled the *M. falcata* in a general way in these respects. The Peruvian and Kansas seed germinated with about the same rapidity and uniformity. The differences, if any, were in

[1] GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. *In* Jour Agr. Research, v. 18, no. 11, p. 553-606, 3 fig., pl. 64-79. 1920. Literature cited, p. 605-606.

favor of the Peruvian. Ten days after the seedlings emerged some differences were evident. These differences increased rapidly, and on March 3 typical plants from each series were removed to the laboratory and photographed. The photographs tell the story. (Pl. 116–121, A.) The seedlings given the normal day which, at this time, was of about the same length as the average October day, behaved in practically the same manner as those grown in the greenhouse from December sowings and the same as those produced from early fall field sowings at Arlington. The seedlings under the light of the short day—from 9 a. m. to 4 p. m.—exhibited the same varietal differences as those under the normal day; but the differences were accentuated, as the photographs indicate. The seedlings grown under the long day—under the day with added illumination from twilight until 11 p. m.—behaved quite differently from the others. The seedlings of *M. falcata* instead of being short and rosettelike in habit, grew erectly with no branching and attained practically as great a height as any of the seedlings in the three series, up to the time photographed. Later on, however, these seedlings were overtaken by the other varieties. The Grimm and Turkestan behaved similarly to *M. falcata;* and, whereas the Peruvian and Kansas seedlings were erect, they failed to keep the relative position with regard to height which they held under the normal and short day. In brief, the abnormally long day produced almost a reversal of order in the matter of height and to a degree a reversal in the habit of growth. The temperature of the greenhouse in which the tests were conducted ranged from 50° to 60° F.; and while the electric light under which the long-day seedlings were placed produced a slight rise of temperature, this did not quite equal the temperature inside of the dark chamber and is considered insignificant so far as its effect on the general results is concerned. The dark chamber was a wooden box 3 by 3 by 3 feet, of ⅜-inch material with light-tight ventilators, top and bottom. (Pl. 121, B.) With the factor of temperature virtually eliminated, it seems fair to conclude that the phenomenon of difference in habit of growth of seedlings of alfalfa varieties obtained both in the field and greenhouse experiments herein described was due, for the most part, to the day-night relation of the varieties.

REACTION OF VARIETIES AND SPECIES TO LENGTH OF DAY IN ACCORDANCE WITH REGIONAL DEVELOPMENT

The evidence is quite clear that Peruvian alfalfa grows best in comparison with others under the relatively short day while the strain of *Medicago falcata* used in these experiments produces its best growth under the relatively long day. It also seems to be true that the Kansas strain resembles the Peruvian in its day-night relations while the Grimm resembles the *M. falcata.* This is not surprising. The Peruvian variety was developed near the Equator where the days are always about 12 hours long. The Kansas, which is the result of several seed generations

grown in that State from original stock probably produced in southern latitudes, must therefore be regarded as a southern strain. The *M. falcata* was from the original stock secured by the Department of Agriculture from eastern Russia. The Grimm strain is a hybrid between *M. sativa* L. and *M. falcata* that has been locally grown in northern latitudes for more than half a century. The Turkestan used in this case behaved much the same as the Grimm; but other lots will doubtless show considerable variation in this respect because of the great range in comparative length of day in different parts of Turkestan. The relation between locality of origin of the various alfalfas and their reaction to the day-night period is shown consistently.

The day-night experiments herein described throw light on some questions that have perplexed students of alfalfa in this country. It is well known that the Peruvian variety in the Southwest continues growth later in the fall than the other commercial varieties and begins growth earlier in the spring. This fact led Brand [1] to conclude that it was able to grow at a lower temperature than the other varieties. He applied the term "zero point" to the minimum temperature at which growth takes place and classed Peruvian alfalfa as a variety with a low zero point. From subsequent investigations it was found that while Peruvian alfalfa continues growth late in the fall and begins it early in the spring in the Southwest and even as far north as Washington, D. C., it does not do so at Redfield, S. Dak., and other northern points. Oakley and Garver [2] were of the opinion that this phenomenon was due to the effect of very low temperature to which the variety was subjected in the North. It is quite clear now that this explanation is not the proper one, for although temperature bears an important relation to growth, it is its day-night relation that causes Peruvian alfalfa to have a longer growing period than the other varieties in the South and to have the same or even a shorter growing period in the North. The northern varieties do not thrive under the short day of southern Arizona and California, but they can more than compete with the Peruvian under the long day of the North. At Redfield the growing season does not extend far into the short days of late fall or winter, neither does it begin in the spring until the long days have arrived. Low temperature in the fall checks the growth of Peruvian alfalfa in the North before the short days give it the advantage over *Medicago falcata*, Grimm, and other northern alfalfas. Low temperature also holds the Peruvian back in the spring until the long days arrive under which the northern alfalfas grow most rapidly. An explanation is now found for the fact that while Grimm alfalfa gives better yields than the Kansas in the North it does not do so as far south

[1] BRAND, Charles J. PERUVIAN ALFALFA: A NEW LONG-SEASON VARIETY FOR THE SOUTHWEST. U. S. Dept. Agr. Bur. Plant Indus. Bul. 118, 35 p., 11 fig., 3 pl. 1907.

[2] OAKLEY, R. A., and GARVER, Samuel. MEDICAGO FALCATA, A YELLOW-FLOWERED ALFALFA. U. S. Dept. Agr. Bul. 428, 70 p., 23 fig., 4 pl. 1917. Literature cited, p. 67–70.

as Virginia. It is an easy matter now to account for the fact that certain lots of imported Turkestan alfalfa give comparatively high yields at Redfield and other northern points while the yields are comparatively low in Virginia and other southern States.

As a result of Garner and Allard's[1] discovery of the photoperiodism of plants it appears necessary to change the recommendations commonly made in regard to the range and adaptation of alfalfa varieties. Instead of recommending the use of southern varieties, such as the Peruvian and Kansas, as far north as they are hardy, these varieties should be recommended for as far north as the length of day during the growing season gives them an advantage over the northern alfalfas that yield comparatively better under the relatively long day. There is a difference between the two types of recommendation, especially along the Washington and Oregon coast and in such areas as the Judith Basin, Mont., where the winters are not so severe as at some other points of the same latitude. Peruvian alfalfa as a rule does not winterkill in the former region, and the Kansas seldom winterkills in the latter. Both sections have a length of day during the growing season that favors the Grimm and its allies. As a matter of fact, the Grimm has given somewhat better yields than the Kansas at Moccasin, Mont. The day-night relation of alfalfa is a much better measure of the adaptability of the varieties than their ability to endure cold and unfavorable winter conditions generally, as is indicated by the fact that Kansas alfalfa is sometimes hardy in the North where the length of the day is less favorable to it than to the Grimm and other northern varieties.

It is believed that by the methods followed by the writers in their recent experiments it will be quickly and easily possible to tell within reasonable limits from a given lot of alfalfa seed in what sections it will give the best yields of hay as compared with other varieties. It may even be possible by this method to determine in a measure to what extent it is winter hardy. Investigations are now being made in this field, and it is confidently expected that a careful study of the day-night relations of alfalfa will throw considerable light on the subject of the development of regional strains.

HOW ALFALFAS MAY BE IDENTIFIED BY SEEDLING CHARACTERS

It is exceedingly difficult if not impossible to identify varieties of alfalfa by seed characters. Where identification is important, as it is in many cases, any short-time method that will assist in accomplishing it is useful. It is generally possible to distinguish between imported and domestic seed by the weed seeds and other incidental impurities contained in the sample. This is particularly true of imported seed of Turkestan alfalfa, but it is practically impossible to distinguish seed of

[1] GARNER, W. W., and ALLARD, H. A. op. cit.

Grimm alfalfa from that of other domestic varieties. Analysts, it is true, become very expert in identifying kinds of alfalfa seed by various pieces of evidence, including weed seeds, dust, and all incidental impurities, as well as by color, size, and percentage of hard seed. But even seed analysts can not always make the critical determinations that are necessary. To the less well-trained person the seed of all varieties of alfalfa is very much alike.

By sowing seed in the greenhouse, as was done in the experiment herein described, it is possible within three or four weeks to distinguish seedlings of one kind from another. If this method is used, it is highly desirable to have on hand viable seed of the most important commercial alfalfas and of at least one strain of *Medicago falcata* to be used in making the necessary comparisons of the seedlings. As a simple method to follow, it is suggested that the seed of the lot or lots to be identified be sown in rows in small flats in which adjoining rows are sown with seed of the Peruvian, Kansas, or some southern-grown common strain, Montana, Dakota, or some northern-grown common strain, Grimm, and a strain of *M. falcata*. For best results there should be three flats, each sown in the same way—one flat to be subjected to a short day, one to an abnormally long day, and one to the normal day as it exists at the time the tests are made. The normal day series may not be necessary, but there are some advantages in including it as a control. It is suggested that the day be shortened for the first series by putting the flat in a room with light excluded or in a box such as is shown in Plate 121, B, at 4 p. m., and leaving it in total darkness until 9 a. m. This gives a sufficiently long day for the necessary photosynthetic action and at the same time it is short enough to produce quick and striking differences in the seedlings of at least certain of the varieties. The length of day may be augmented for the second series by using a tungsten bulb of 100 watts and a reflector as shown in Plate 121, B. It is suggested that the light be turned on at dusk and turned off about 11 o'clock p. m. This gives an abnormally long period of constant illumination, but such a period is helpful in producing striking results quickly. By the use of both the short and the long day the differences between the northern and southern alfalfas are better shown. The normal-day series adds evidence when the differences of the seedlings are studied.

While it is not possible by this method to distinguish between certain closely related varieties or strains such as the Grimm, Baltic, and Canadian variegated, these strains as a group can be positively distinguished from the common and other commercial strains by the general habit of growth and lack of uniformity of the seedlings. While the Turkestan seedlings can easily be distinguished from the Kansas by their height and lack of uniformity, they resemble those of the Grimm closely enough to make positive identification difficult. Since there is great diversity in the various lots of Turkestan seed that are imported commercially,

it is probable that weed seed and other impurities in the seed will be more satisfactory than the seedling characters as a means of identification. The regional strains of common alfalfa may be distinguished from the Peruvian, on the one hand, by their more rapid very early growth under the long day and from the Grimm, Baltic, Canadian variegated, and Turkestan, on the other, by their greater height, erectness, and uniformity under the short day. Table I sets forth briefly the characteristics of the seedlings of the various alfalfas under the different lengths of day as determined by experiments conducted in the greenhouse at Washington, D. C.

TABLE I.—*Characteristics of seedlings exposed to varying periods of light daily. Sown in greenhouse January 20; notes taken on March 3, 1921*

Varieties.	Exposed to normal day with electric light from dusk to 11 p. m.	Exposed to normal day.	Exposed to daylight from 9 a. m. to 4 p. m.
Peruvian	Erect, no branching, height 5 inches.	Erect, no branching, height 3⅗ inches.	Erect, no branching, height 3 inches.
Kansas[a]	Erect, no branching, height 4 ½ inches.	Erect, no branching, height 3¼ inches.	Erect, no branching, height 2½ inches.
Grimm	Erect, no branching, height 4⅘ inches.	Low and spreading, stipules enlarged, basal branches just showing, height 2½ inches.	Low and spreading, rosette-like, stipules enlarged, basal branches just showing, height 2 inches.
Turkestan	Erect, no branching, height 4⅘ inches.	Low and spreading, stipules enlarged, basal shoots just showing, height 2 inches.	Low and spreading, rosette-like, stipules conspicuous, basal shoots just showing, height 1⅗ inches.
Medicago falcata	Erect, slender, no branching, height 5 inches.	Low and rosette-like, stipules conspicuous, height 1¼ inches.	Low and rosette-like, stipules conspicuous, height 1 inch.

[a] These measurements were taken too late to show the proper relation between seedlings of the Kansas and those of the Peruvian.

By the use of this method and by the aid of such facts as can frequently be had regarding the lots of seed to be identified, it will be found possible in many cases to make a reasonably certain varietal identification. The method is offered especially to Experiment Station workers and those engaged in the administration of alfalfa seed certification activities as an aid to and not as a positive means of identifying varieties. It is recognized that the work herein reported upon is far from complete, but investigations are now being conducted to make it more comprehensive and useful.

The method involving the sowing of seed in the field is not recommended where a greenhouse is available. It is less certain and its use is limited to sections where the short days of the year are sufficiently warm to produce growth.

SUMMARY

When seed of Peruvian, Kansas-grown common, Grimm, and Turkestan alfalfa are sown in the early fall in the approximate latitude of Washington, D. C., the seedlings develop characteristics that make it easily possible for the casual observer to distinguish one variety from another. Seedlings from spring sowings did not develop differences at Washington or in northern latitudes in the United States that would be apparent to the untrained eye. Seedlings from greenhouse sowings in December at Washington showed essentially the same distinguishing characters that were shown by early fall sowings in the field at Arlington.

At first it was concluded that the temperature of the fall days was responsible for the striking differences shown by the alfalfa seedlings. However, the results of Garner and Allard's work on the effect of the length of the day on the growth of plants suggested the advisability of growing alfalfa seedlings in the greenhouse under artificially lengthened and also under artificially shortened days.

Three series of small flats were sown with seed of Peruvian, Kansas, Grimm, and Turkestan alfalfas, and *Medicago falcata* on January 20, 1921. One series was allowed to have the effect of the normal day, one was given the effect of a day shortened by the use of a light-excluding box placed over the flat at 4 p. m. and allowed to remain until 9 a. m., and one was given the effect of a day lengthened by turning on an incandescent electric light at dusk and turning it off at 11 p. m. The seedlings grown under the normal and the shortened day behaved essentially the same as those from sowings made in the early fall at Arlington. In height, erectness, and lack of branching the varieties ranged as follows: Peruvian, Kansas, Grimm, Turkestan, *M. falcata*. Seedlings under the lengthened day showed striking differences between the varieties but behaved in practically the reverse order from those under the normal and the shortened day. At the end of the first month the seedlings of *M. falcata* and Grimm exceeded those of the Peruvian and Kansas varieties in height. They were also quite as erect and unbranched.

From these experiments it is evident that it is the day-night relation of alfalfas and not their reaction to temperature that causes the development of marked differences between the seedlings of the various varieties when seed is sown at certain times of the year. By the proper control of the length of day, which can be accomplished in the greenhouse at any time of the year with little trouble or expense, and by the method used in these experiments it is easily possible to distinguish between seedlings of the commercial groups of alfalfas. The tests can be so quickly and easily made that the method is offered especially to Experiment Station workers and those engaged in alfalfa seed certification work as a means of assisting in the identification of the various lots of seed.

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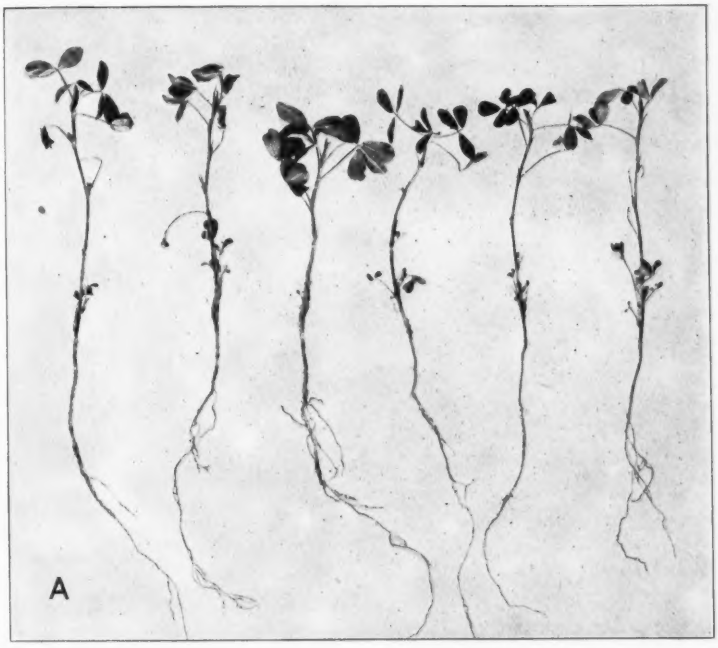
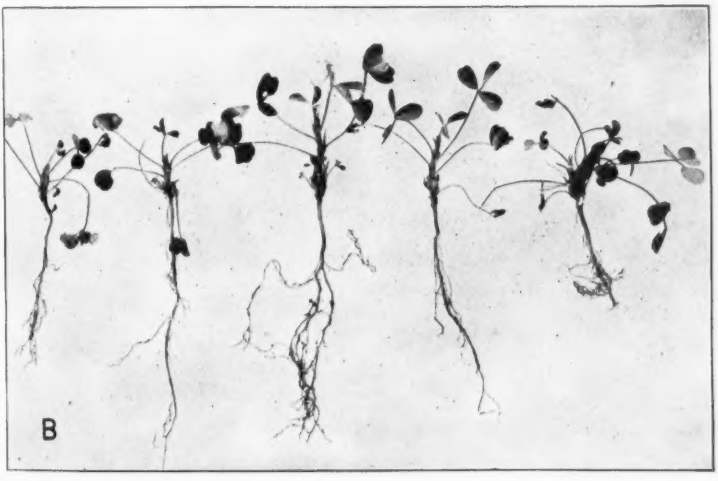
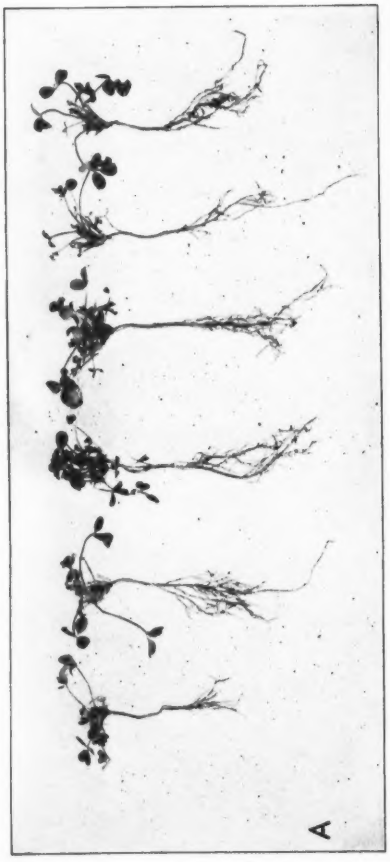
PLATE III

A.—Peruvian alfalfa sown at Arlington, Va., September 6. Photographed December 5, 1916.

B.—Kansas alfalfa sown at Arlington, Va., September 6. Photographed December 5, 1916.


A


B


A

B

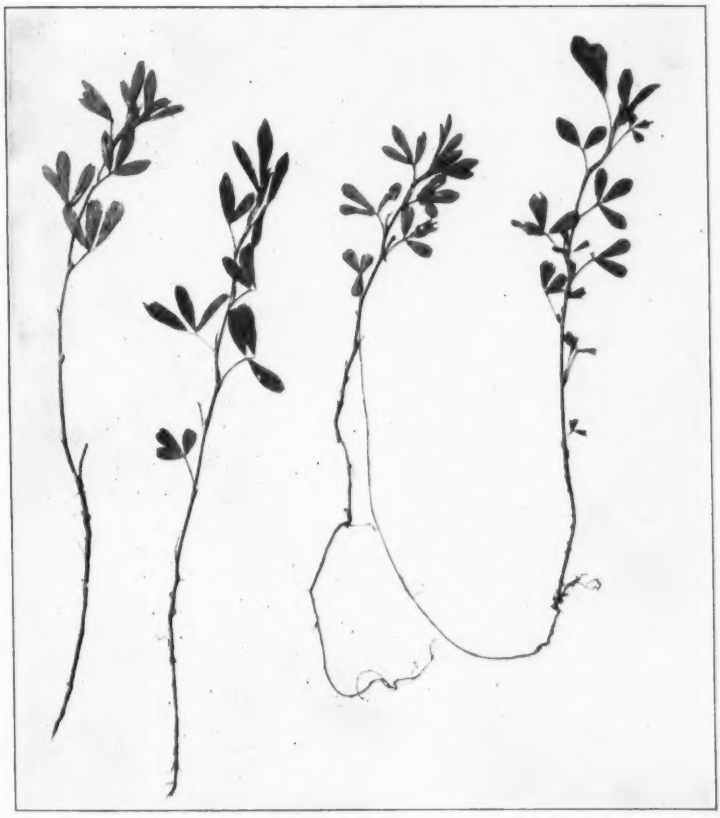
PLATE 112

A.—Grimm alfalfa sown at Arlington, Va., September 6. Photographed December 5, 1916.

B.—*Medicago falcata* sown at Arlington, Va., September 6. Photographed December 5, 1916.

PLATE 113

Peruvian alfalfa sown at North Ridgeville, Ohio, May 3. Specimens taken July 1, 1919.



Effect of Length of Day on Seedlings of Alfalfa

PLATE 114

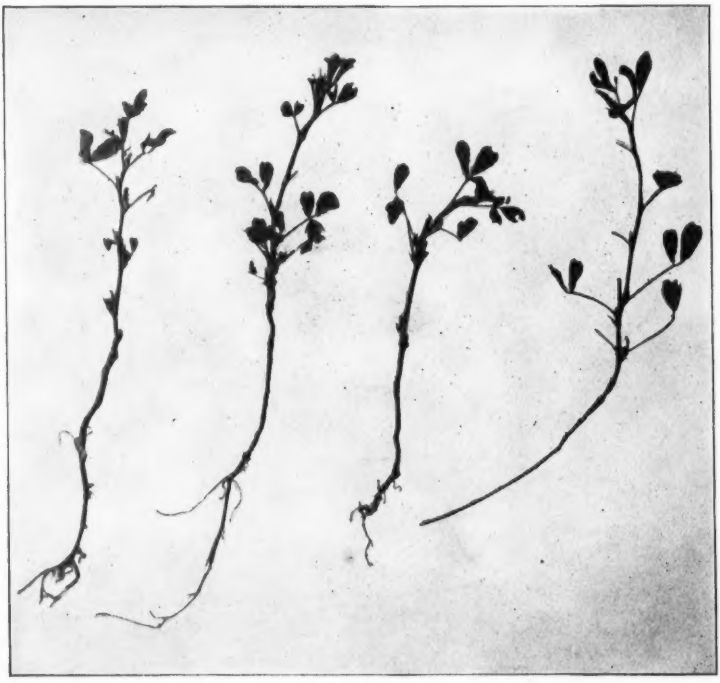
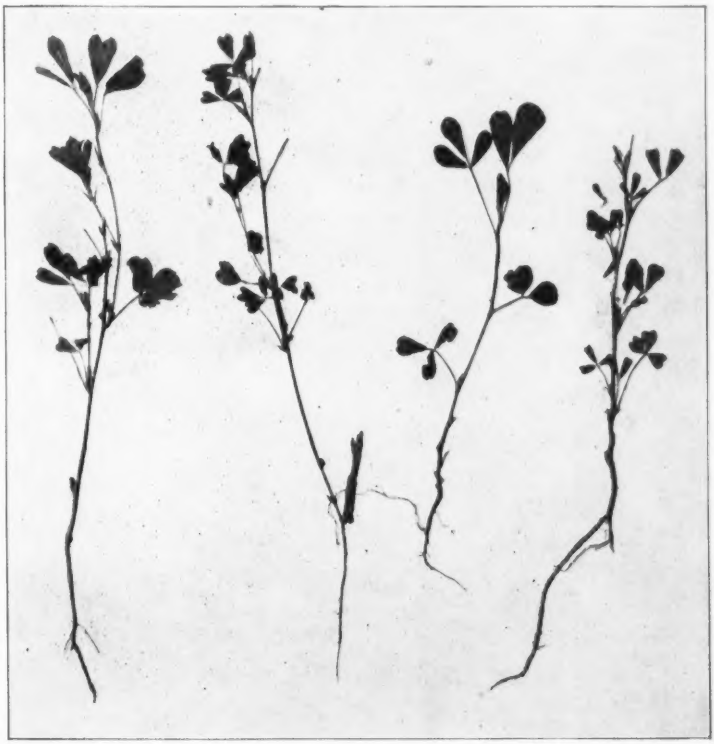


PLATE 114

Kansas alfalfa sown at North Ridgeville, Ohio, May 3. Specimens taken July 1, 1919.

PLATE 115

Grimm alfalfa sown at North Ridgeville, Ohio, May 3. Specimens taken July 1, 1919.



A

B

C

PLATE 116

Peruvian alfalfa sown in greenhouse January 20. **Photographed March 3, 1921.**

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

B.—Grown under normal day.

C.—Exposed to daylight from 9 a. m. to 4 p. m.

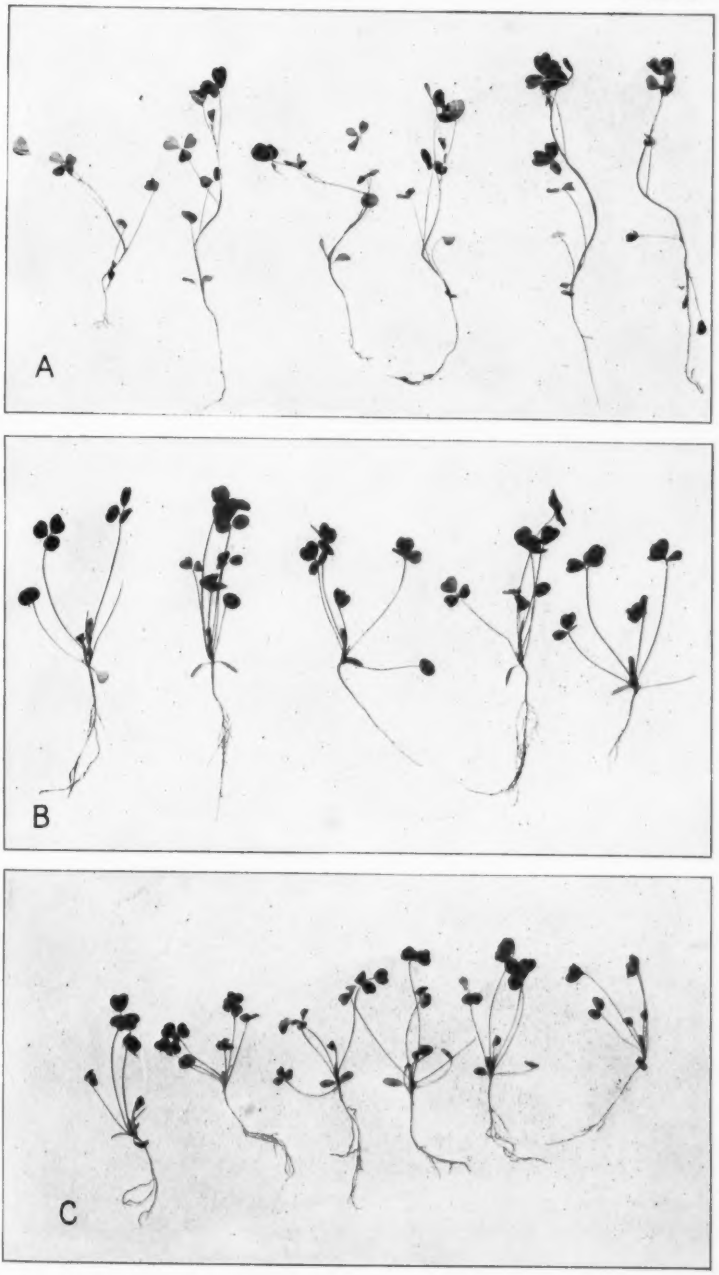
PLATE 117

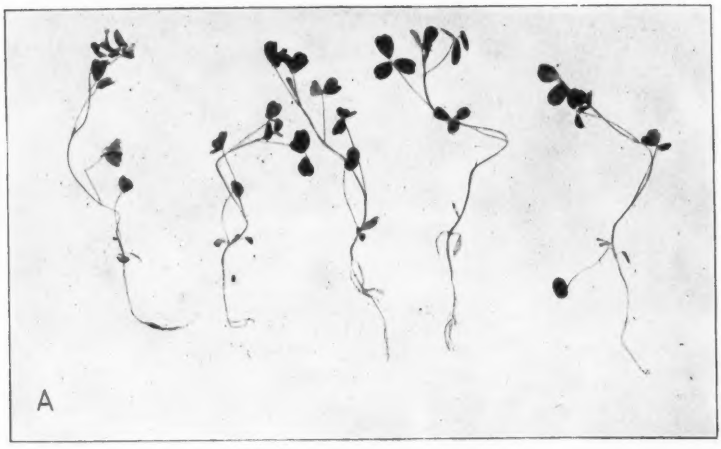
Kansas alfalfa sown in greenhouse January 20. Photographed March 3, 1921.

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

B.—Grown under normal day.

C.—Exposed to daylight from 9 a. m. to 4 p. m.


A
B
C


A

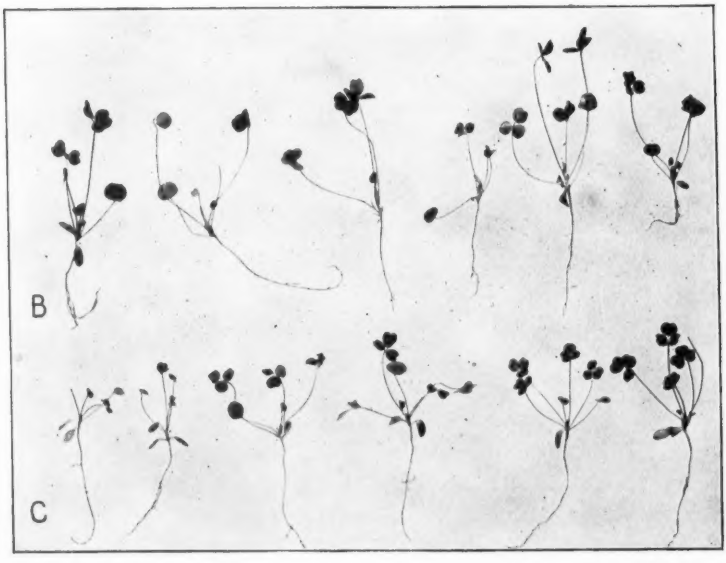

B
C

PLATE 118

Turkestan alfalfa sown in greenhouse January 20. Photographed March 3, 1921.

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

B.—Grown under normal day.

C.—Exposed to daylight from 9 a. m. to 4 p. m.

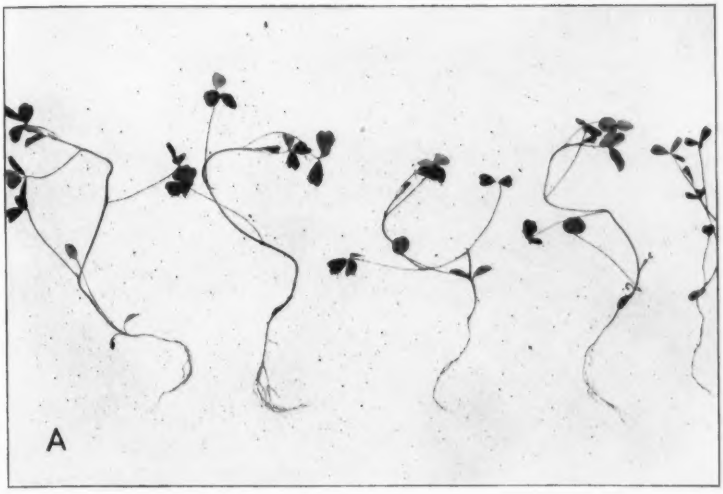
PLATE 119

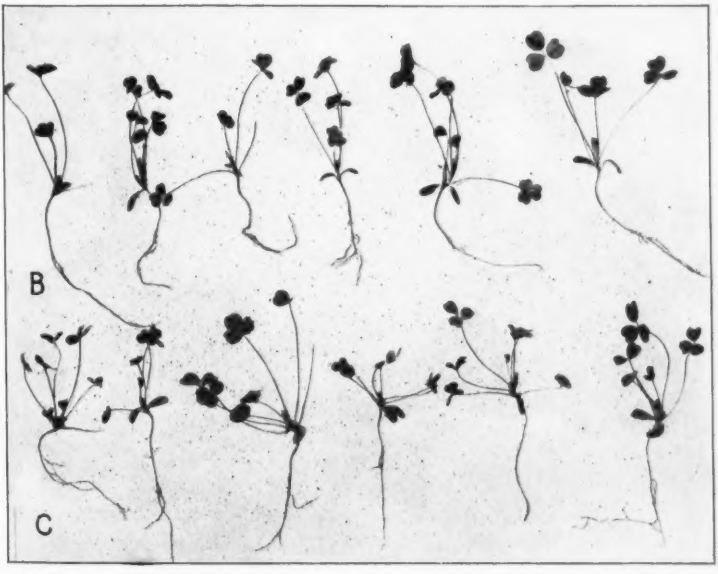
Grimm alfalfa sown in greenhouse January 20. Photographed March 3, 1921.

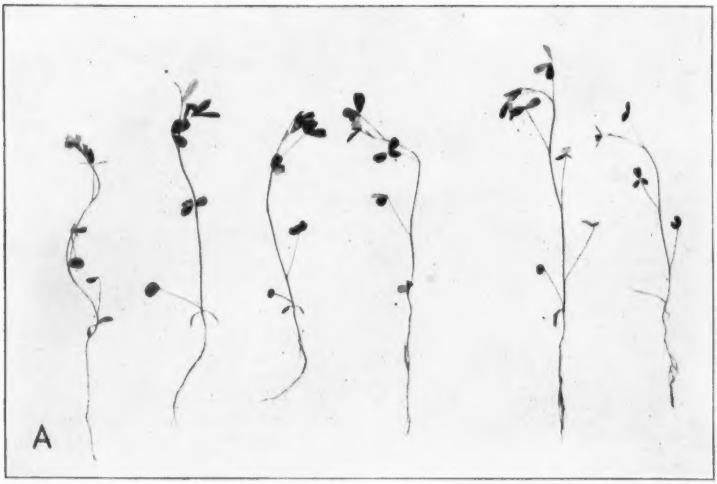
A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

B.—Grown under normal day.

C.—Exposed to daylight from 9 a. m. to 4 p. m.


A


B
C


A

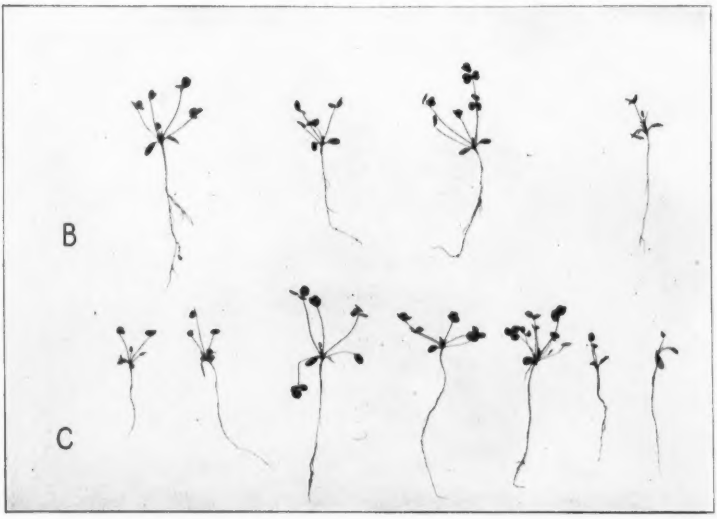

B
C

PLATE 120

Medicago falcata sown in greenhouse January 20. Photographed March 3, 1921.

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

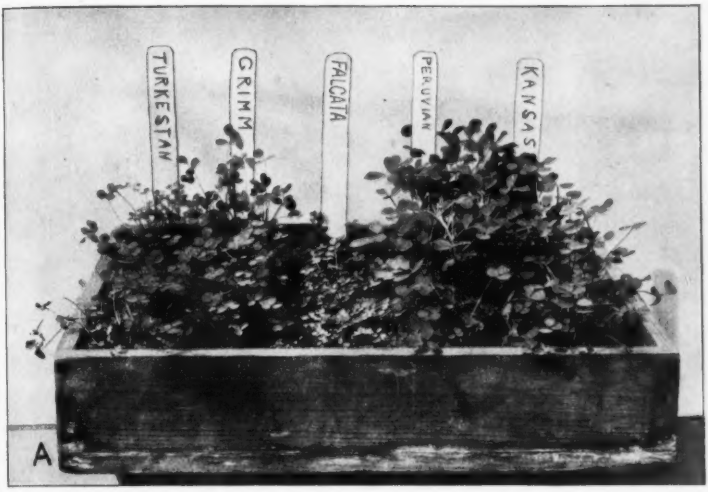
B.—Grown under normal day.

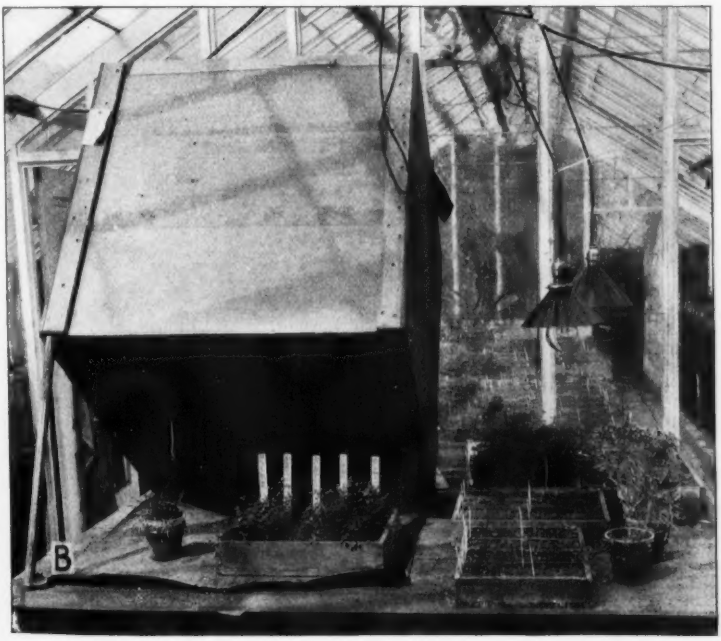
C.—Exposed to daylight from 9 a. m. to 4 p. m.

PLATE 121

A.—Alfalfa seedlings showing the effect of a short day on the growth of different varieties. Seed was sown in the greenhouse January 20, and seedlings were exposed to light from 9 a. m. to 4 p. m. Photographed March 22, 1921.

B.—Equipment used in determining the effect of different lengths of day on the seedling characters of alfalfa varieties.


TURKESTAN
GRIMM
FALCATA
PERUVIAN
KANSAS
A


B